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Assistant Editor:
MARTIE E. HAMILTON, B.Sc.

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Vol. 17. MAY, 1944. No. 2

Experiments on the Effect of Nitrogenous Manures on the Yield of Garden Peas at Dickson, A.C.T.

*By E. M. Hutton, B.Ag.Sc., M.Sc.**

Summary.

Under the conditions of these experiments, sulphate of ammonia applied at planting did not significantly increase the yield of green peas, whereas this manure and nitrate of soda applied at flowering at the rates of 224 lb. and 288 lb. per acre respectively significantly increased the yields by 15·1 per cent. and 12·6 per cent.

1. Introduction.

Little information is available about the effect of the application of nitrogenous manures on the yield of peas. Cornell (1932) failed to obtain any significant increases in the yield of garden peas with sulphate of ammonia applied at seeding. Musbach and Sell (1936) found that complete fertilizers containing nitrogen, phosphorus, and potash increased the yield of garden peas when compared with fertilizer treatments involving phosphorus and potash or phosphorus alone.

2. Comparison of the Effects of Nitrogen Applied at Seeding and at Flowering on the Yield of Garden Peas.

The experiments described in this paper were planted early in August, 1943, on an area at the Division's Experimental Farm at Dickson, A.C.T. The area had been limed several months earlier with hydrated lime at the rate of 2 tons per acre. A week prior to sowing superphosphate was drilled in at the rate of 2 cwt. per acre. The pea seed was treated with the appropriate Rhizobial culture a few hours before the seed was sown.

The treatments were replicated eight times and the plots randomized. Each plot was planted at inch intervals and contained 400 seeds; the distance between plots was 5 links. The amounts of sulphate of ammonia applied to the various plots at planting were distributed two weeks before seeding along open furrows which were filled in and then re-opened before the pea seed was planted. Showers of rain at this period ensured an even distribution of the sulphate of ammonia. The amounts of sulphate of ammonia and nitrate of soda applied to the plots at flowering were dissolved in water and distributed evenly along furrows

* Vegetable Research Officer, Division of Plant Industry, Canberra.

on both sides of the row of plants. The furrows were then filled in. At flowering, sulphate of ammonia was used at the rate of 224 lb. per acre and nitrate of soda at the rate of 288 lb. per acre, as these quantities contain approximately equivalent amounts of nitrogen.

The field emergence percentages with the sulphate of ammonia applied at planting were 91 for no application, 93 for 2 cwt., 75 for 5 cwt. and 61 for 10 cwt. The field emergence percentages of the plots which were sown at the same time for the application of nitrogenous manures at flowering averaged 93. All the plots grew well during the season, except those receiving the heavy applications of sulphate of ammonia at planting, and these were retarded early in the season but grew vigorously later.

TABLE.—YIELDS OF GREEN PEAS IN THE POD FOR THE VARIOUS TREATMENTS WITH NITROGENOUS FERTILIZERS.

(Yields in lb.)

Treatment.	Nitrogen Applied as Sulphate of Ammonia at Planting.	Nitrogen Applied at Flowering.	
		Sulphate of Ammonia.	Nitrate of Soda.
Nil	20·30	17·90	17·62
2 cwt.	18·52	20·60	19·84*
5 cwt.	20·16	..	..
10 cwt.	9·22	..	..

Minimum differences for significance—

(1) At 5 per cent. point for N at planting 3·71.

(2) At 1 per cent. point for N at flowering 2·10.

* 288 lb. of nitrate of soda applied.

There are no significant yield differences between no application and 2 cwt. and 5 cwt. of sulphate of ammonia applied at planting. The yield from the 10 cwt. of sulphate of ammonia was significantly less than the others, probably because of the poorer field establishment and the excessive vegetative growth of these plots. The application of nitrogenous manures at flowering significantly increased the yield, sulphate of ammonia by 15·1 per cent. and nitrate of soda by 12·6 per cent. There was no significant difference between the increases resulting from the two manures. Pod measurements from all plots demonstrated that none of the treatments affected pod size. Root nodulation appeared to be normal in all plots.

3. Acknowledgments.

The statistical analysis of the data was made by Mr. G. A. McIntyre. The Biological Branch of the New South Wales Department of Agriculture kindly supplied the Rhizobial cultures used in the experiments.

4. References.

Cornell, H. H. (1932).—*Farming S. Afr.* 7: 65-6.

Musbach. F. L., and Sell, O. E. (1936).—*J. Agr. Res.*, 53: 869-879.

The Field Emergence and Yield of Garden Peas as Affected by Treatment of the Seed with Fungicidal Dusts.

*By E. M. Hutton, B.Ag.Sc., M.Sc.**

Summary.

1. Under the conditions of these experiments spergon was the only fungicidal dust of those tried which consistently and significantly increased the field emergence of garden peas.

2. Spergon markedly improves the percentage field emergence from poor quality pea seed, but has only a small effect on good seed. The William Massey samples used gave on the average lower field emergences than Greenfeast, so that William Massey received the most benefit from dusting with spergon.

3. None of the fungicidal dusting treatments tried significantly increased the yield of garden peas.

1. Introduction.

Of recent years there has been a tendency to replace mercurial and copper dusts used on pea seed with non-metallic organic compounds, such as spergon, thiosan, and fermate (McNew, 1943). Spergon is the best known of these, and Sharvelle *et al.* (1942) and Crosier (1943) have reported increases of 21-25 per cent. in the field emergence of peas as a result of treating the seed with it. Sharvelle *et al.* (1942) obtained an average increase in yield of 18 per cent. by treating pea seed with spergon.

In Australia, pre-emergence damping off in peas is common in many soils, so that seeding rates of 3 to 4 bushels per acre are often necessary to obtain a satisfactory stand of plants. Pea seed, therefore, figures as one of the main costs in the canning crop. This investigation was made to compare the effects of dusting seed with spergon, ceresan, and cuprox on field emergence and yield in the varieties Greenfeast and William Massey, in the hope of being able to reduce seeding rates and thereby ease the strain on seed supplies.

2. The Effect of Dusting on Field Emergence.

Two separate field experiments were designed to test the effect of seed dusting on field emergence. The first involved the use of spergon, ceresan, and cuprox dusting on two very good samples of William Massey and Greenfeast. The second experiment covered 50 samples of William Massey and Greenfeast from different districts in Australia and New Zealand and involved dusting with spergon only.

The first experiment was carried out in two localities at Canberra, A.C.T., on widely differing soil types. The first soil type at Black Mountain was of poor quality, and the second one at the Division's Experiment Station, Dickson, was a good clay loam. Both areas received lime at the rate of 2 tons per acre earlier in the year, and, just prior to sowing, superphosphate at 2 cwt. per acre. The plots were seeded early in October, 1943. Inoculation of the pea seed with the appropriate

* Vegetable Research Officer, Division of Plant Industry, Canberra.

Rhizobial culture was given in the evening, and the next morning the samples for the various plots were dusted with the fungicides and then sown at inch intervals in single row plots. The plots on Soil I. were sown with 280 seeds, and those on Soil II. with 400 seeds. The distance between plots was 5 links. Each treatment was replicated 8 times and the plots randomized.

TABLE 1.—PERCENTAGE FIELD EMERGENCE OF GARDEN PEAS ON TWO SOIL TYPES AFTER TREATMENT WITH FUNGICIDAL DUSTS.

Dust Treatment.	Dusts Applied at 2 oz. per Bushel.			
	William Massey.		Greenfeast.	
	Soil I.	Soil II.	Soil I.	Soil II.
	%	%	%	%
Spergon	65·04	76·37	78·75	87·19
Ceresan	49·06	66·88	66·96	83·16
Cuprox	52·01	62·59	76·83	83·53
Control	44·87	64·19	64·73	83·09

Minimum differences for significance—
Soil I. at 5 per cent. point = 8·24.
Soil II. at 5 per cent. point = 2·84.

Table 1 gives the results for the four treatments. The plant counts from which the field emergence percentages were calculated were made at the first leaf stage. These results demonstrate that under the conditions of the trials spergon was the only dust treatment consistently and significantly better than the untreated controls. On the average it gave a 12 per cent. increase in the field emergence. Neither the ceresan nor the cuprox treatments were significantly better than control, except in the one case with Greenfeast on Soil I. when cuprox was as good as spergon.

The seeding of the second experiment involving the 50 pea samples from different sources was commenced towards the end of October, 1943. Samples were sown at monthly intervals three times. At every sowing two pairs of plots, one dusted with spergon and the other undusted, were planted from each of the samples. A plot consisted of 120 seeds sown at inch intervals. The plots were randomized. Plant counts were made at the first leaf stage and the percentage field emergence determined.

TABLE 2.—MEAN FIELD EMERGENCES OF UNDUSTED AND SPERGON-DUSTED SEED FOR THE THREE SOWINGS.

Monthly Sowing.	Spergon-Dusted.		Undusted.		Increase.	
	I.*	II.	I.	II.	I.	II.
	%	%	%	%	%	%
October	80·24	..	71·69	..	8·55	..
November	78·09	79·35	66·25	66·45	11·84	12·90
December	76·80	76·33	65·23	65·05	10·57	11·28
January	..	78·27	..	64·85	..	13·42

* For the purpose of statistical analysis the data were treated in two sets. The first set was planted on the first three dates and the second set on the last three dates.

Table 2 shows that the mean percentage field emergence for the fifty samples varied from one planting to the next, the tendency being towards a poorer field emergence as the season advanced from the cool spring conditions to those of the hot summer. The difference between dusted and undusted seed did not vary significantly from one sowing to the next.

Table 3.—The Effect of Spergon on Samples with Differing Field Emergences.

Number of Samples.	Mean Percentage Field Emergence from Three Sowings of Undusted Seed.	Mean Increase in Percentage Field Emergence Due to Spergon.
12	Less than 60	18
20	60–80	12
22	Greater than 80	4

A summary of the increases obtained as a result of dusting seed of different quality with spergon is given in Table 3. These results demonstrate that the field emergence of the poorer seed can be improved, so that it approaches that of good seed, whereas the best seed is not benefited to any marked degree by dusting with spergon. Examination of the data showed that for samples of different variety or origin having the same field emergence when undusted, the increases due to spergon do not vary significantly. However, the results in Table 4 show that on the average the William Massey samples were considerably poorer than the Greenfeast, and their field emergences were benefited to a greater degree by dusting with spergon.

Table 4.—Showing the Mean Field Emergences for the Three Plantings of the William Massey and Greenfeast Samples Used.

Number of Samples.	Variety.	Mean Percentage Field Emergence.	
		Spergon-Dusted.	Undusted.
		%	%
17	William Massey ..	69·32	53·10
33	Greenfeast	88·04	83·29

Variety difference significant at the 1 per cent. point.

With this series of samples the differences in favour of the Greenfeast are of the order of 30 per cent. for the undusted and 20 per cent. for the spergon-dusted seed.

This tendency for William Massey samples to give a poorer field emergence than Greenfeast needs further investigation. That this does not always apply is evidenced by the results of Norris and Hutton (1943). However, both the experiments cited in the present paper indicate much better field emergences from Greenfeast than from William Massey—a fact unexplained by the visual examination of the seed samples used.

3. The Effect of Seed Treatment with Fungicidal Dusts on the Yield.

The plots which were sown to compare the effects of spergon, ceresan, and cuprox on the field emergence of Greenfeast and William Massey were grown to maturity and harvested as green peas in the pod during December, 1943. During the growing season there were no visible differences in vigour apparent among the plots, although soon after field emergence there appeared to be a slight difference in favour of the spergon treatments. The yield results indicated that none of the dust treatments had significantly increased yield over the undusted controls.

4. Acknowledgments.

The statistical analyses of the data were made by Mr. G. A. McIntyre. Dr. J. R. A. McMillan supplied the pea samples from the various districts in Australia and New Zealand. The Biological Branch of the New South Wales Department of Agriculture generously supplied the Rhizobial cultures used in the experiments.

5. References.

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The Reaction of Varieties of *Trifolium subterraneum* to Attack by *Uromyces trifolii* as a Heritable Character.

*By K. Loftus Hills, B.Agr.Sc.**

Summary.

The commonly grown variety of subterranean clover, known as Mt. Barker, is very susceptible to leaf rust disease. Other varieties, particularly those of the early maturing type, are not attacked. Mt. Barker has been crossed with the early variety Mulwala and it has been shown that resistance to the disease is an inherited character, although early maturity tends to be associated with resistance. However, both early and late segregates have been obtained in the third generation of the cross which are immune from attack and which possess to a large extent the agronomic characters of the parents.

1. Introduction.

It has been shown by the writer† that the different varieties of *Trifolium subterraneum* vary greatly in their reaction to attack by the organism (*Uromyces trifolii*) causing leaf rust.

* An officer of the Division of Plant Industry.

† The Reaction of Varieties of *Trifolium subterraneum* to Leaf Rust (*Uromyces trifolii* (Hedw.) Lev.) *J. Coun. Sci. Ind. Res.* (Aust.), 15: 272-4 (1942).

There is a tendency for the earlier varieties to be free from the disease, but both resistant and susceptible varieties are found among the later-maturing types. The common mid-season variety, Mt. Barker, is very susceptible to attack by the rust pathogen, while the early variety Mulwala appears to be immune. If these varieties are crossed it should be possible to determine from the behaviour of the progeny whether resistance to the disease is transmitted, and whether the earliness of a genotype influences its resistance. At the same time, it should be ascertained whether improved, rust-resistant varieties could be produced from such a cross.

2. Material and Methods.

In 1941 four F_1 plants were raised from seed produced by crossing the varieties Mulwala and Mt. Barker.‡ Seed from one of these plants, together with that of the parents, was sown in flats in the autumn of 1942, and the resulting seedlings transplanted into the field as spaced plants, arranged in ten randomized blocks. At the end of the season there were 160 plants of Mt. Barker, 223 of Mulwala, and 331 of the F_2. General observations were made from time to time on each plant of vigour, leafiness, etc., and a record was kept of the date of the appearance of the first flower on each plant, notes being taken for the purpose twice weekly throughout the flowering period. An epidemic of leaf rust occurred during October, and notes were made on the infection of each plant according to the following scale:—

0. No rust pustules visible.
1. An occasional single pustule.
2. Moderate infection of most leaves.
3. Heavy infection of practically all leaves.

Seed was harvested from all the F_2 plants, but owing to war-time circumstances, only 22 of the most promising non-infected plants could be carried on to F_3. Seedlings were raised in the nursery as before and transplanted into the field on a check row system, groups of three F_3 progeny rows alternating with either parent. There were 25 plants in each F_3 family. General observations were made as in the previous year, but at flowering each plant was allotted to one of seven maturity groups according to the proportion of open flowers present at successive inspections. Temperatures during spring were unusually low. No sign of rust infection was noticed until the beginning of December, when Mt. Barker plants began to show leaf rust pustules. At the middle of December, detailed notes were made on infection in a manner similar to the previous year.

3. Results and Discussion.

The rust infection ratings for F_2 plants and the parents are summarized in Table 1. The susceptible parent was characterized by a uniform and heavy infestation, practically all leaves on all plants being thickly covered by rust pustules. By contrast, the resistant parent did not show a single lesion. The F_2 plants fell principally into two groups corresponding closely to the parents, but, in addition,

‡ By Dr. J. R. A. McMillan in 1940.

TABLE 1.—RUST INFECTION OF PARENT AND F_2 PLANTS.

	Rust Infection Rating—			
	0.	1.	2.	3.
Mulwala	223	..	..	..
Mt. Barker	..	..	..	160
F_2	96	9	17	205

TABLE 2.—THE RUST INFECTION, FLOWERING BEHAVIOUR, AND AGRONOMIC VALUE OF F_2, F_3, AND THE PARENTS.

Progeny.	Rust Infection Rating—		Maturity.		Mean Agronomic Rating F_3 8 : 7 : 43.
	F_2 Plant.	F_3 Family (Mean).	F_2 Plant* (Flowering Day)	F_3 Family† (Mean of Flowering Group Ratings).	
Mt. Barker ..	3 (mean of 160)	2·1	269	6·0	2·1
Mulwala	0 (mean of 223)	0	237	1·4	2·1
TS–1361	0	0	234	1·4	2·1
TS–1363	0	0	234	2·4	1·8
TS–1366	0	0	238	1·4	1·0
TS–1368	0	0	238	1·9	2·7
TS–1369	0	0	234	1·2	2·4
TS–1373	0	0	234	1·5	2·0
TS–1374	0	0	234	2·4	2·0
TS–1375	0	0	238	1·6	1·8
TS–1376	0	0	234	2·7	1·8
TS–1378	0	0	238	..	1·3
TS–1381	0	0	238	2·1	1·9
TS–1382	0	0	246	3·3	2·0
TS–1360	0	0	268	3·8	1·7
TS–1362	0	0	261	2·5	1·7
TS–1364	0	0	265	5·4	1·9
TS–1365	0	0	265	6·0	1·6
TS–1367	0	0	261	5·4	2·3
TS–1371	0	0	265	4·4	1·8
TS–1372	0	0	265	4·2	2·3
TS–1377	0	0	265	5·3	1·9
TS–1379	0	0	272	5·8	1·8
TS–1380	0	0	272	5·8	2·1

* March 1st = 60, hence day 234 is August 22.

† Group 1 is the earliest, 7 the latest.

there were small numbers of plants which could not properly be included in the major groups, but which appeared to be minor modifications of them. Thus, the nine plants classed as "1" were somewhat similar to the "0" group, but both "0" and "1" were for all practical purposes free from the disease whilst "2" and "3" could be described as moderately to heavily infected.

The behaviour of the progenies of the uninfected F_2 plants which were carried on to F_3 is shown in Table 2. Although the epidemic of rust was a month or more later than in the previous year, all the Mt. Barker plants were attacked to some extent, although the average rating was less. Mulwala was again quite free from the disease. All the progenies of uninfected plants were, themselves, uninfected. In columns 4 and 5 of the same table are shown the flowering times of the F_2 plants and their F_3 progenies. F_3 families TS-1365 and TS-1380 are as late flowering as Mt. Barker and yet are apparently quite immune to rust attack. The remaining families in the later group contain a proportion of plants as late as Mt. Barker which are also resistant to the disease. It is clear that the rust resistance of the early parent has been combined with the mid-season maturity of Mt. Barker.

The data are inadequate to establish definitely the mode of inheritance of rust resistance, but it would appear that susceptibility is dominant. If, in the F_2 data, the classes 0 + 1 and 2 + 3 are combined, we obtain two groups corresponding roughly to susceptibles and resistants with a ratio of 223 to 108. The probability that this represents a ratio of 3:1 is less than 1 in 100 ($\chi^2 = 10{\cdot}273$). In Table 3, the F_2 data have been arranged in the form of a contingency table of rust reaction against flowering day. The tails of the flowering-day frequency distribution have been grouped to facilitate analysis. The value of χ^2 is 27·84 so that the probability that the two characters are independent is less than 0·001. Examination of the table indicates that the earlier classes contain a higher proportion of resistant plants than expected on the basis of an even distribution. This linkage may be either genetic or environmental, or possibly both may play a part. If the assumption is made that the discrepancy in infected plants in the early groups is due to their escaping infection, then the table may be divided into two parts, those plants which commenced to flower on or before day 251 and those which did so subsequently. In the former section the ratio of infected to non-infected plants is 58:59 and in the latter 164:50. The probability that the latter represents a ratio of 3:1 is high ($\chi^2 = 0{\cdot}305$ and $P > 0{\cdot}5$). Such a ratio would be explained by the fact that the inheritance of resistance is controlled by a single pair of factors, the parents being constituted SS and ss respectively, and susceptibility being completely dominant. There is a high probability that some at least of the early susceptible group which presumably escaped infection in the F_2 were included in the F_3. But as no infected plants occurred, it would have to be assumed that conditions were such as to preclude infection of early types entirely. Although the rust epidemic was much later in the second year, it seems unlikely that there would be such a complete absence of infection in material which was still green. It is possible that a genetic linkage of earliness and rust resistance is the explanation of the association found for the F_1 material as a whole.

From the agronomic point of view, the F_3 plants fall into two groups, early rust-resistant types comparable in form and behaviour to Mulwala, and mid-season rust-resistant types similar in general form to Mt. Barker. Each plant was allotted a rating on a 1-5 scale according to its size, leafiness, and general attractiveness, a grading of 1 being the smallest and poorest type. The mean family values for the observation are set out together with that of the parental checks in column 6, Table 2. A number of desirable Mt. Barker type plants did not appear as attractive during the post-flowering period, being rather less leafy than the Mt. Barker plants, but there were some exceptions. The early types included a proportion of plants which were more attractive than Mulwala right through their life history, being leafier and more vigorous.

TABLE 3.—THE RELATION OF RUST REACTION AND MATURITY IN THE F_2 OF MULWALA X MT. BARKER.

Flowering-day Class.	Rust Reaction—	
	Resistant.	Susceptible.
234–238	13 (8·2)*	12 (16·8)
239–246	16 (10·1)	15 (20·9)
247–251	29 (19·9)	32 (41·1)
252–257	30 (37·8)	86 (78·2)
258–261	10 (21·5)	56 (44·5)
262–280	10 (10·4)	22 (21·6)
Total	108	223

$X^2 = 27·84$. $P < ·001$.

March 1st = 60, hence day 234 is August 22

* Figures in brackets refer to the number of plants expected in each class if maturity and rust reaction are independent.

4. Conclusions.

1. The resistance of *T. subterraneum* to attack by *U. trifolii* is an inherited character.

2. Susceptibility is dominant, the progeny of uninfected F_2 plants being free from rust.

3. There is evidence of linkage between time of flowering and rust resistance, but it has not been proven whether this is genetic or environmental. However, it is of a low order, and combinations of earliness and susceptibility, and of lateness and resistance, occur frequently.

4. Several resistant F_3 lines were of the same maturity as Mt. Barker and very similar to that parent in general form and appearance. It is probable that a variety could be evolved from the cross which would combine the desirable agronomic characters of Mt. Barker with the rust resistance of Mulwala.

An Investigation of *Myxomatosis cuniculi* with Special Reference to the Possible Use of the Disease to Control Rabbit Populations in Australia.*

By L. B. Bull, D.V.Sc., and M. W. Mules.

1. Introduction.

The investigation was started by Sir Charles Martin in 1934. His work was of a preliminary nature and was carried out at the Institute of Animal Pathology, University of Cambridge. His results were published in Bulletin No. 96 by the Council in 1936, and they outline the findings of four colony experiments. These were carried out in a relatively small netted compound 50 yards long by 10 yards wide. A summary of the results as recorded in the Bulletin is as follows:—

(*a*) *Experiment* 1.—A virus of low virulence received from Dr. Aragao, Rio de Janeiro, was used. The disease was introduced into a colony of 27 tame rabbits. After 89 days, 25 of the rabbits had died and two recovered from the infection.

(*b*) *Experiment* 2.—The same virus of low virulence was used and was introduced into another colony containing 25 tame rabbits. After 65 days, all the rabbits and their progeny had died with the exception of one.

(*c*) *Experiment* 3.—A virus of higher virulence received from the Rockefeller Institute for Medical Research was used, and was introduced into a colony of 55 wild rabbits. After 41 days the whole colony was exterminated.

(*d*) *Experiment* 4.—The same virus of higher virulence was introduced into a colony of 44 wild rabbits. After 35 days, the whole colony was exterminated.

In addition, contact experiments were carried out in cages. Of the rabbits contracting the disease due to the virus of low virulence, 4 per cent. recovered. Only one rabbit out of 208 infected with the virus of higher virulence recovered.

A small amount of work was done on the specificity of the virus to supplement the information in the literature at that time. Five cattle and five sheep were inoculated with large doses of virus suspension and in no case did any of these animals show any evidence of infection or any departure from normal health.

As these preliminary experiments showed that a strain of the virus of reasonably high virulence would exterminate a population of rabbits in a circumscribed area, and as all the evidence showed the virus to be specific for the European rabbit and its domesticated varieties, it was deemed advisable and safe to continue the investigation in Australia.

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* This is a brief report of the investigation. The full account and presentation of scientific data will be published later in the Council's Bulletin series.

The virus material was received in Melbourne in August, 1936. Its origin was in South America but it had been kept by the Rockefeller Institute for Medical Research under standard conditions for ten years up to the time it was taken to Cambridge. These conditions had been maintained up to the time it was sent to Australia.

2. Laboratory Experiments.

(i) *Specificity of the Virus.*

In America rabbits belonging either to the genus *Sylvilagus* or to the genus *Lepus* have been found to be refractory. The European rabbit belonging to the genus *Oryctolagus* has been found to be consistently susceptible by all workers. Sheep, goats, horses, pigs, cattle, dogs, cats, fowls, pigeons, ducks, man, monkey, guinea-pigs, mice, rats, ferrets, and hamsters have all been tested by workers overseas, and all have been found to be refractory.

This work was repeated in Australia, and in addition sixteen varieties of native animals were tested. All these animals were found to be refractory. These results have been published.*

(ii) *Enclosure Experiments.*

Two enclosure or colony experiments have been carried out in Australia with wild rabbits. The enclosure was completely netted on the sides and top in order to keep out predatory animals, such as cats, and birds. It had an area of 400 square yards in which two natural warrens were established.

First Colony Experiment.—This experiment was started with 38 wild rabbits. During the first few days five deaths occurred from fighting and other non-specific causes. When the rabbits had settled down an infected rabbit was placed in the compound. A rabbit dead of myxomatosis was found above ground 16 days later. The disease spread slowly, and in 51 days the colony was exterminated. Of the rabbits in the enclosure after the introduction of the infected animal, 28 were found dead above ground and were proved to have died from myxomatosis. The remaining five rabbits died underground, presumably from myxomatosis.

Second Colony Experiment.—The object of this experiment was to determine what might happen over a period of time if normal healthy rabbits were added periodically to an infected colony. A colony of 28 rabbits had become stabilized and on 6th February, 1937, an infected rabbit was added to it. A second infected rabbit was added on 12th, and a third on 16th. All three infected rabbits died of the disease between 13th and 18th.

Normal rabbits were then added to the colony, at first slightly irregularly and later six were added quite regularly twice a week until 27th August, 1938. In all, 965 rabbits were passed through the colony.

Towards the end of the experiment coccidiosis occurred in the colony. On account of this infestation the rabbits when they contracted myxomatosis died in the acute stage of the disease before they could pass it on to contacts. This brought about the elimination of

myxomatosis in the colony. The last death from myxomatosis occurred on 10th August, 1938, in one of the batch of six added on 27th July. The colony was broken up on 5th September by catching the 44 rabbits that remained. These were inoculated with a large dose of virus suspension in order to determine if any had developed an immunity. Two of the rabbits were found to be resistant and must be regarded as having recovered either from a subclinical or clinical infection. One had been in the colony since 29th December, 1937, and the other since 12th January, 1938.

During the course of the experiment some rabbits died from causes other than myxomatosis, in others the cause of death remained doubtful, and some rabbits died in the warrens, thus preventing any investigation of the cause of death. Although mating occurred and aborted foetuses were found at times, no viable progeny was found in the colony.

Between 6th February, 1937, and 23rd July, 1938, a total of 851 rabbits were added to the colony and 703 were found dead. Of these deaths, 612 were from myxomatosis, 65 were non-specific, and 26 were from doubtful causes. Most of the non-specific deaths occurred during the first week of colony life. Most of the deaths from myxomatosis occurred about the twelfth day of colony life, although a few were delayed beyond the 50th day and up to the 141st day.

There was some fluctuation in the length of life in the colony or, in other words, in the "cage-age" at death during the experiment, but at the end of the experiment it was no less than at the beginning. On this basis there was no loss of virulence during the experiment.

The evidence thus shows that in a confined space the disease may be maintained indefinitely in a colony when fully susceptible rabbits are added to it at regular intervals, provided that other epizootic diseases are excluded.

Recovery from myxomatosis occurred in two rabbits out of 929 exposed to infection between 6th February, 1937, and 10th August, 1938.

(iii) *Insect Transmission Experiments.*

Wild rabbits in Australia are commonly infested with species of *Echidnophaga,* the native stick-fast flea, especially during the warm months of the year. As these fleas and also mosquitoes form part of the environment of rabbits in the wild state in Australia, experiments were carried out to determine if any of these insects are capable of acting as vectors of the virus.

(*a*) *Experiments with Echidnophaga myrmecobii as vector.*—For the purpose of the experiments, the fleas were bred in the laboratory. The first experiments were made to determine if the fleas will leave the carcass of a diseased rabbit and seek a new host. Two experiments were carried out, and in each it was found that the fleas left the carcass of the infected host and a proportion of them found and attached themselves to a new host which developed myxomatosis in seven days. Another experiment showed that all fleas would not leave the carcass early, but batches could be found to leave from time to time up to 36 hours after the death of the host. Fleas leaving an infected host in

16 hours, 27 hours, and 36 hours, conveyed the disease to new hosts in every instance. It was also determined that fleas having fed on the first infected host would transfer to a second host and to a third host. In this way, fleas that had been feeding for 20 days on infected hosts were still sufficiently active to transfer to and infect a third host.

Fleas leaving one host are able to travel some distance in seeking a new host. It was found that if fleas leave the infected host voluntarily after its death they are capable of transmitting the disease up to three days later. However, if the fleas are irritated to induce them to detach, they do not carry the infection to the new host. Flea larvae fed on flea faeces containing virus were found to harbour the virus, presumably in the gut, but when they became adults they did not transmit the disease. No modification of the virus was observed when it was given eleven passages by fleas through rabbits.

These and other observations showed that the flea *Echidnophaga myrmecobii* can act as a vector of the virus. Transmission of the virus by the flea is by so-called mechanical means. There is no evidence of cyclical development in the flea.

(*b*) *Experiments with Mosquitoes as Vectors.*—*Aedes aegypti* is easy to breed, to feed, and to handle under laboratory conditions, and therefore it was used mainly in the experiments. *A. alboannulatus, A. notoscriptus, A. camptorhynchus,* and *Culex fatigans* were also used, but to a more limited extent.

The mosquitoes were studied singly in some instances and in groups in other instances. They were fed on infected rabbits about the time the disease became generalized or within two days of this. The virus is to be found in the blood of infected animals about two days before generalization occurs and for two or three days after.

The experiments showed that *Aedes aegypti* readily transmitted the virus immediately after a feed on an infected rabbit and for about fourteen days subsequently. It would transmit the disease with more certainty by the first feed on a normal rabbit after the infective feed. However, it might continue to infect normal rabbits in subsequent feedings. Sometimes there would be failure of transmission for no apparent reason. The disease was also transmitted under similar conditions by *A. alboannulatus, A. camptorhynchus,* and *Culex fatigans.* One experiment with *A. notoscriptus* failed to give transmission of the disease. No significance can be attached to this single observation. It is believed that most mosquitoes would transmit the disease much in the same way as *Aedes aegypti* under natural conditions. Possibly transmission would depend more on the voracity of the mosquito than on its genetical make-up.

(iv) *Contact Experiments.*

All workers have found that usually the disease is readily conveyed to healthy rabbits coming in contact with diseased rabbits after generalization has occurred. We have been unable to obtain infection in healthy rabbits by contact with infected rabbits during the incubation period.

The disease has been transmitted by contact in a continuous series for many months. On rare occasions a contact of this sort will fail to produce infection, although the contact rabbit can be shown subsequently to be fully susceptible to artificial inoculation of the virus.

The virus escapes from the body mainly in the discharges from the eyes and from the nose. If lesions develop in the skin, discharges from these contain the virus. It has not been demonstrated in the urine and faeces.

Under natural conditions of contact, infection appears to be by the respiratory route, although experimentally it can be brought about by placing infective material in the conjunctival sac. When precautions are taken in handling experimental rabbits, accidental cross-infections can be very rare but are difficult to prevent completely. Occasionally an accidental cross-infection may occur. It appears that this may be due to droplet infection or to virus particles attached to dust particles carried in convection currents in the air. The infectivity appears to increase with an increase of the discharges from the nose and eyes. It appears to be low in the early generalized stage of the disease. A rabbit usually sniffs the face of another when coming into contact with it. This habit possibly leads to insufflation of infective material even in the moist state and possibly accounts for the majority of the infections that are contracted under natural or colony conditions.

If actual contact is not established, infection is not transmitted very readily. Infected rabbits may sneeze, although this has not been observed to occur commonly in rabbits under laboratory conditions. Experiments have shown that if an infected rabbit and a healthy rabbit are placed face to face and held within an inch or less from nose-tip to nose-tip, infection may occur if the exposure is prolonged for about 24 hours. It rarely, if ever, occurs if exposure is reduced to an hour or two. If the rabbits are confined in a tunnel in which a steady stream of air is moving constantly and in which the air moves over the infected to the healthy rabbit, infection is facilitated and made more certain. In one experiment the rabbits were held in pillories in the tunnel, facing one another, and 3 inches apart from nose-tip to nose-tip. In four out of thirteen experiments rabbits contracted infection under these conditions. This suggests that infective material may escape from the surface or in the breath of an infected rabbit, but it may be dispersed quickly in convection currents.

As the result of many observations it is concluded that the communicability of the disease under natural conditions is not of a high order, although under laboratory conditions a small proportion of chance infections may occur, and taken alone they may suggest a high communicability. Infectivity, which is usually quite low during the first day of manifest external signs of the disease, increases with the escape of discharges on to the body surfaces.

(v) *Artificial Inoculation by a Trap Device.*

All workers have found that very small doses of virus when injected artificially into the tissues of a rabbit will establish an infection. It has been estimated by some that a single virus particle is capable of establishing infection on artificial inoculation. Ingestion of virus does not produce the disease, but it is readily produced through small wounds in the skin.

In order to find a possible means of introducing the virus into a colony of rabbits living in warrens under natural conditions, experiments were carried out with the ordinary rabbit trap modified in such a way that it would wound and inoculate the rabbit but not catch or trap it.*

There is a vast accumulation of knowledge on the use of the ordinary rabbit trap for the purpose of catching rabbits. The knowledge on the best method of setting and placing the trap in relation to the warren or the "playground" could be applied to the use of the modified trap for the purpose of infecting rabbits with the virus.

Laboratory experiments showed that a large proportion (75 per cent.) of rabbits springing the modified trap charged with a virus suspension became infected.

As the trap has to be set in the surface soil some hours before it is expected that the rabbit will visit it, and as the surface soil reaches a high temperature during the day in the warm months of the year, observations were carried out on the actual temperatures to be found in the first inch of soil. Observations at the State Research Farm, Werribee, showed that the maximum soil temperature at 1 inch may reach 129·4°F. The soil temperatures at 1 inch, taken at Koonamore, S.A., during a warm cloudless day without wind, reached 143°F. at 2 p.m. when the shade air temperature was 103°F.

The virus in suspensions of infective tissue is reasonably resistant, but it will not withstand temperatures of 100°F., or more for very long. It is obvious, therefore, that soil temperature may be a limiting factor in the application of the trap inoculation method of introducing the disease into rabbit colonies.

3. Field Experiments.

(i) *Wardang Island—First Experiment.*

The small enclosure experiments had shown that the disease is capable of exterminating a colony of rabbits in a confined space. It could not be assumed that the disease would spread in the same way under natural conditions.

The first field experiment was carried out on Wardang Island, in Spencer's Gulf, about 3 miles from the mainland of Yorke Peninsula, South Australia. The experimental area of approximately 90 acres was enclosed by a wire-netting fence and surrounded by another wire-netting fence to give a buffer area 3 feet wide. Foxes, dogs, and other predatory animals did not exist on the island.

At the commencement of the experiment the enclosure contained fourteen well-established warrens, some being in the open and others more or less under low shrubs. There were no trees, and there was no natural water in the enclosure.

The experiment was designed to answer the following questions:—(*a*) Will "foreign" rabbits infected and introduced into a particular area become accepted by the established colonies sufficiently quickly to enable the disease to be introduced and to spread through them? (*b*) Will the disease when established in a colony spread to contiguous

* *J. Coun. Sci. Ind. Res.* (Aust.), **15**: 82-3 (1942).

colonies? If so, what may be the limit of range? (*c*) What normal inter-communication takes place between colonies and what factors favour contact between individuals from different colonies?

The rabbit population in the enclosure was estimated at between 400 and 500. Conditions were dry and the surface was covered by long dry grass. Small water troughs were placed at ground level in several positions in the enclosure. The rabbits drank between 10 and 13 gallons of water nightly.

On 16th November, 1937, twenty rabbits which had been caught outside the enclosure were inoculated with virus into the conjunctival sac, were earmarked for identification, and were liberated in the south-west corner of the enclosure. Most of the introduced inoculated rabbits did not join the established colonies, but two or more did join colony No. 2, one of a group in the south-west corner.

The first "enclosure" rabbit to be seen dead of myxomatosis was found eighteen days after the introduction of the disease. From this time onwards, rabbits dead from myxomatosis were found in increasing numbers. The peak was reached about 50 days after the introduction of the disease, and by the 100th day 238 rabbits had been found dead above ground. The last freshly dead rabbit was found on 22nd February, 98 days after the introduction. The disease may be said to have died out about this time.

The population was densest in the south-west corner of the enclosure, where the disease started in Colony No. 2 and possibly in one or two other colonies, although this could not be determined with certainty. In all, five colonies became definitely infected and all were situated close together in the south-west corner.

Rabbits dead of the disease were found in all parts of the enclosure, but the density was greatest in the south-west corner. Obviously the sick rabbits had wandered aimlessly away from their colonies. This tendency of the sick rabbit to separate itself from the colony and from other colonies lessens the chance of spread in a colony and from it to other colonies.

Evidence could not be found of recovered rabbits, and there was nothing to suggest that the disease died out because of the development of a resistant population of recovered rabbits.

(ii) *Wardang Island.—Second Experiment.*

After the conclusion of the first experiment the rabbits in the enclosure were allowed to increase naturally. The population was also built up by the addition of 530 rabbits from outside. By the end of May, 1938, the population was estimated to be about 1,000 adults with an unknown number of kittens. New warrens had been established bringing the number to 33. A large number of the rabbits were living above ground apart from the warrens.

The disease was re-introduced on 28th June, 1938, by trapping in wire-netting traps at the warrens and inoculating the rabbits caught. These rabbits were earmarked for identification and returned to their own warrens. In all twelve adult rabbits and 21 kittens were inoculated at nine warrens.

Only seven of these inoculated rabbits were found dead of myxomatosis. During July and the first three days of August, seventeen uninoculated rabbits were found dead of the disease. The disease appeared to die out or to fail to reach much momentum. Further inoculations were therefore made during August. In all, 51 adult rabbits and 168 kittens were inoculated at ten warrens.

During the period from 1st August until 29th October the numbers of rabbits found dead of myxomatosis for each ten-day period were, 0, 3, 69, 82, 80, 92, 75, 87, 84, respectively. The death rate fell off in November to 41, 22, and 24. An attempt was made to increase the death rate and spread of the disease by further inoculations. On 30th November, 32 rabbits were inoculated at water trough A; on 2nd December, 29 at trough C; on 5th December, 3 at trough B, and on 6th December, 21 at trough B, making 85 in all. The death rate increased for a time, but fell again.

Natural feed in the enclosure had become exhausted and hand-feeding with oats was carried out from 13th November, 1938, to 27th January, 1939, but as the death rate had fallen again and conditions had become so unnatural through the necessity to hand-feed, the experiment was then terminated by poisoning the remaining rabbits, 1,078 in all.

In the seven months of the experiment, only 934 uninoculated rabbits were found; others would have died in the warrens.

In summary, the figures are as follows:—

337 Rabbits were inoculated in all, on four occasions.
934 Uninoculated rabbits died of myxomatosis.
633 Rabbits died from heat and starvation.
1,078 Rabbits remained in the enclosure and were killed by poison or other means.

(iii) *Point Pearce.—Third Experiment.*

The two experiments in the enclosure on Wardang Island failed to show that the disease would set up an epizootic that would exterminate a rabbit population of either small or high density in an area of 90 acres. In an area of this size the rabbits have greater freedom of movement than in an enclosure of 400 or 500 square yards such as was used in the so-called laboratory experiments. This freedom of movement allows the infected rabbits to separate themselves from their colonies, thus removing the source of infection. This instinct to isolate themselves from the rest of the herd is exhibited by many species of animals.

If the sick rabbit left an infected insect vector in the warren or dropped infected vectors in its wanderings, the epizootic might have much better chances of being maintained and of spreading.

Rabbits on the mainland are commonly infested with species of the native stick-fast flea, *Echidnophaga*. The flea was not present on rabbits inhabiting Wardang Island, nor did we succeed in an attempt to establish it on rabbits in the enclosure. As the flea had been proved to be capable of acting as a vector of the virus under laboratory conditions it became necessary to determine if the disease would behave differently in a rabbit population carrying flea infestation than in one free from fleas.

A new enclosure of 90 acres was established on the mainland at Point Pearce. The rabbits inhabiting the surrounding area of country were infested with stick-fast fleas. The colony was therefore built up with naturally infested rabbits caught with the aid of ferrets. This infestation was supplemented with laboratory-bred fleas. There were thirteen large warrens in the enclosure. By December, approximately 500 rabbits were present and all the warrens were occupied.

The experiment was started between 11th and 15th December by catching 28 rabbits in netting traps at six warrens in the centre of the enclosure. The skin on the nose of these was scarified and inoculated with glycerinated virus suspension. Each rabbit was earmarked for identification. Of the inoculated rabbits, ten were found recently dead of the disease in ten to fifteen days after inoculation. The rabbits in five of the six inoculated colonies became infected from the primary inoculation. Infection spread to the other colonies.

As foxes were present in the neighbourhood, precautions had to be taken to keep them out of the enclosure. Their presence influenced the behaviour of the rabbits which kept beneath the ground during most of the hours of daylight, making observations more difficult than on Wardang Island.

By 28th January, 1941, four of the warrens were completely deserted. Another warren became deserted shortly afterwards. Sick rabbits were seen up to 13th February. Observations were continued but no further sick or dead rabbits were found. The experiment was terminated on 2nd March by catching the remaining rabbits. From five warrens a total of seventeen rabbits were obtained. The numbers in the warrens were 4, 4, 2, 2, and 5, respectively. These rabbits were inoculated with virus, and all were found to be susceptible and died of the disease, except one which died of some intercurrent disease during the incubation period.

In this experiment the disease spread to all warrens in the enclosure and exterminated a population of approximately 500 rabbits, with the exception of seventeen, in 60 days. It is concluded that the spread of the disease within the colonies and from colony to colony was greatly assisted by the presence of an insect vector, the native stick-fast flea. This result is in marked contrast with the results obtained in the two experiments on Wardang Island with rabbits free from flea infestation.

4. Field Trials.

Although the field experiments in enclosures of about 90 acres did not give encouraging results in the absence of an insect vector, the investigation could not be regarded as being complete in the absence of trials carried out under natural field conditions free from restraint on the movement of rabbits and under the full play of all the ecological factors present in a selected environment.

The field experiments had shown that the disease failed to spread from warren to warren with sufficient ease to establish a momentum which would reduce the density of the population within reasonable time. However, it was judged practicable to overcome this defect by a systematic spread of the disease among the warrens by the mechanical

means of the modified rabbit trap. Further, experience had indicated that once the disease entered a colony it could be expected to destroy the majority of its members. This was at least true for the early colony experiments.

Particular attention was given to the study of the rabbit pest in the semi-arid bush country to be found in the north-east portion of South Australia in which the destruction of natural bush and herbage cover has led to serious erosion of the surface soil.

Under favourable seasonal conditions the rabbit populations can reach great density in this country. For example, over a period of about three years seasonal conditions had favoured the propagation of the rabbits, and commercial firms had found it profitable to trap the rabbits over this area for the supply of rabbit carcasses to the market. The peak period was reached early in 1940, when on Koonamore Station 3,000 rabbits were being placed in the freezer each day.

The average annual rainfall is approximately 8 inches in this area, but in 1940 only about 1·26 inches of rain fell. The rabbits died off very rapidly through lack of water, and by the end of the year few or no rabbits were to be seen on Koonamore and the adjoining stations of Melton and Mount Victor.

It is normal for droughts of this nature to occur periodically and for the rabbit populations to be virtually exterminated owing to lack of water. Obviously this extremely low level in rabbit population density can be taken as a base line. If the density of the population could be prevented from again increasing to a level of plague proportions the problem constituted by the rabbit pest would be solved. If periodic introductions of myxomatosis at critical times could succeed in lowering the population density, then a valuable aid in the control of the rabbit pest would be available.

The field trials were, therefore, designed to determine if this method of attack might succeed. We did not believe that the right time for attack was when the population density had become high, because previous experiments had shown that myxomatosis caused greater relative destruction in a relatively stabilized population of moderate density and because a delayed attack would permit much damage to plant cover by the large number of rabbits. It was not until October, 1942, that the rabbit population on Mount Victor had reached a density sufficiently high to justify the commencement of a field trial. The areas of the four stations on which the work was carried out are, Mount Victor 195 square miles, Koonamore 206 square miles, Melton 428 square miles, and Holowiliena 274 square miles.

(i) *Field Trial at Mount Victor.*

(*a*) Warrnambool paddock was selected as the area for the first trial. It had an area of 3,125 acres approximately. A wide watercourse ran through it from south to north, and varied in width from about 50 chains at the southern end to 75 chains at the northern end. The watercourse provided an area of good feed for the rabbits which were living in warrens along its eastern and western edges.

The eastern side was chosen for the area to be trapped and the western end was left undisturbed as a control. There were 62 warrens on the eastern side which were all well-populated, especially those at the southern end where there was permanent water. Foxes were known to be present; as many as thirteen were counted at the watering place at one time. Crows and eagles were numerous.

The trapping of the eastern side was commenced on 29th November and concluded on 9th December, 1942. The traps were set at 62 colonies and at three patches of bush. In all 344 traps were set, and of these 279 were sprung overnight. The operations were conducted during the evening when the soil temperatures had fallen to a safe level. Between 10th and 17th December, sick and dead rabbits were found. On 5th January, 1943, it was observed that eight of the warrens showed a decrease in activity due to depletion of the colony population. One colony was deserted and three others almost completely deserted. The rabbits kept very much underground and were difficult to observe. During this period only six dead and three sick animals were found. No sick or dead rabbits were found after the 18th December, and it appears that the disease died out between this date and 12th January.

It was concluded that the disappearance of sick and dead rabbits was due to the action of predatory animals, especially foxes. The quick removal of sick rabbits from around the warrens possibly was largely responsible for the quick disappearance of the disease.

(*b*) It was decided to repeat the trial but to reduce the fox population first if possible. During the period from 12th January to 29th March, 1943, a large number of poison baits were laid. In all, 41 foxes, five eagles, and a large number of crows were known to have been killed, and probably the actual number was greatly in excess of this. Heavy rain fell on 17th February and the watercourse ran, especially on the western side. This greatly improved the feed and many of the rabbits migrated to the western side.

It was decided to make the second trial on the western side. There were 46 colonies in which the population was estimated to be 672, and these were selected for trapping. Seven warrens were selected for control purposes and were left uninoculated. Trapping was commenced on 17th March, 1943, and was concluded on 19th March. In all, 213 traps were set at the 46 warrens and 176 of these were sprung overnight. Sick rabbits were not seen until 29th March; five were seen between this date and 6th April. Only three dead rabbits were found up to 7th April. After this time no change was observed.

Again the disease had failed to become established and to have any appreciable effect on the rabbit population. It appears that the attempt to reduce the fox population by the use of poison baits had failed to have any appreciable effect.

(*c*) Following this second failure to establish the disease an intensive campaign was carried out against the foxes. Many baits were taken and some foxes were found poisoned. The rabbit population had remained very much the same as during the second trial. Trapping was commenced on 17th May and was concluded on 19th May. In all, 144 traps were set at 33 colonies, and of these 78 were sprung overnight.

The area was carefully inspected on 24th May, but sick animals could not be found. From 13th June to 19th June the colonies were inspected each day, but diseased or dead rabbits could not be found. Tracks of foxes were numerous on the warrens and many nests had been dug out of the warrens by the foxes.

The disease did not become established, and the introduction had no effect on the rabbit population.

(ii) *Limited Field Trial at Koonamore.*

The rabbit population had not increased to the same extent on Koonamore. However, in one localized area about 500 rabbits were established within a radius of 1 mile of a well at which they were watering. A netting yard was placed around the well and 103 adult rabbits were caught in this, inoculated subcutaneously with the virus, and then liberated. The object was to determine by retrapping at the well if the population had been decreased.

Unfortunately, rain fell six days later and the rabbits ceased to water at the well. The area around the well was thoroughly examined, but at no time were sick or dead rabbits found. The inoculated rabbits probably developed the disease but evidently an epizootic failed to develop, possibly due to dispersion and redistribution of the rabbits after the rain, as well as to the removal of sick rabbits by foxes and cats.

(iii) *Field Trials at Melton.*

(*a*) The density of the rabbit population at Melton had remained at a low level, but in December, 1942, an area containing active colonies was found. These occupied an isolated patch of eleven warrens within an area of about 80 acres extending over a strip a little less than half a mile long by between three and four hundred yards wide. Of the eleven active warrens, nine were chosen for an attempt to introduce the disease into each by means of the trap. It was estimated that from 20 to 40 rabbits occupied the individual warrens, giving a total population in the nine warrens of approximately 250. Thirty-two traps were set, between two and five being set at each warren. Of these, 23 were sprung.

A careful examination eighteen days after trapping showed that five of the warrens were completely deserted, two contained one rabbit, one contained two, and one contained eight rabbits. This means that the population was reduced from approximately 250 to twelve within eighteen days. Of the two control colonies one contained fifteen and the other twenty rabbits. It would appear, therefore, that the disease had reduced the population of the nine warrens appreciably.

A further examination was made twenty days later, i.e., 38 days after the trapping. Rabbits still persisted in the colony which twenty days previously had contained eight, and the control colonies were still fully active. The disease had apparently died out and had not extended to the control colonies. These three colonies were therefore inoculated by the use of traps, and observations carried out twenty days later showed that the warrens were deserted.

The results show that the trap had succeeded in introducing the disease into each of the nine warrens and that the rabbits had been completely or almost completely destroyed in all except one, in which the population had been reduced from 40 to eight before the disease died out. A reinoculation of this warren, as well as inoculation of the two control colonies, succeeded in eliminating the rabbits from all the warrens. Earlier examination had shown that flea infestation of the rabbits was not apparent. The area was apparently free from foxes at the time. Crows and hawks, however, were active, especially in attack of the carcasses of rabbits dead of the disease.

(*b*) A thick population of rabbits was found early in January surrounding a watering place known as Wright's dam. These were in open plain country mainly east and west of the dam. The rabbits were watering at the dam. There was adequate feed for them on the plains and no migration to or from other feeding grounds took place during the experiment. Foxes were watering in small numbers at the dam at the commencement of observations. Poison baits were laid and evidence of the presence of foxes disappeared. Crows and eagles were seen frequently, and a small number of wild cats lived in the area.

An imaginary line was drawn through the centre of the dam. The area to the west, 340 acres, was selected for an attempt to introduce the disease by the use of the trap and the area to the east, 180 acres, was reserved for control observations. In the experimental or trapped area there were 30 colonies with an estimated population of 488 rabbits. In the control area there were 35 colonies with an estimated population of 401 rabbits.

Examinations showed that stick-fast fleas (*E. myrmecobii*) were present in large numbers on the rabbits throughout the experimental area.

Trapping to introduce the disease into the colonies was carried out for three days from 18th January, 1943. As the weather was hot, the traps were set during the day, but the virus capsules were not fitted until towards evening. At the 30 colonies, 150 traps were set, and 114 of them were sprung. On the eighth day after the setting of the last trap, sick rabbits were seen about some of the warrens and along the dam drain. One rabbit recently dead of the disease was found on a warren. Observations were continued up to 6th February, and on each occasion sick or dead rabbits were seen. On 14th February all the warrens were examined and activity was found to be greatly decreased; one warren was completely deserted and another almost completely deserted; five recently dead animals were seen near three of the colonies. On 21st February, 32 to 34 days after trapping, fourteen warrens were found to be completely deserted, nine to be almost completely deserted and containing two or three rabbits, and seven to be partly deserted. No sick or recently dead rabbits were found, and it is probable that the disease had died out. Throughout these observations the warrens in the control area showed evidence of great activity. At no time did the disease extend from the trapped area.

In this trial a rabbit population of approximately 488 was reduced to about 60 in 32 to 34 days. There seems to be little doubt that the infestation of the rabbits with stick-fast fleas contributed to the spread of the disease within the colonies. The epizootic was not modified by the action of predatory animals.

(*c*) The absence of a high fox population at Melton had apparently favoured the promising results obtained in the two small trials. Over most of the station, however, the rabbits had failed to increase, mainly due to the continued dry weather and the absence of green food.

By May, 1943, there was an appreciable increase in the rabbit population in an area of about 7 square miles known as Chinaman's paddock, which was well-grassed flooded land. The rabbit warrens were situated mainly on rising ground along each side of a wide watercourse. The rabbits were breeding. A number were shot and searched for fleas. Although they were found on most of the rabbits, the numbers were too small to be of any significant value in the spread of the disease.

At 1st June, 1943, foxes were present in fairly large numbers. They had apparently been attracted from the neighbouring country to the area by the increasing rabbit population. Poison baits were laid, and although a number were taken, there was no appreciable decrease in the fox population during the period of the observations. Just before this time, 200 foxes had been poisoned in this area in a period of seven weeks. In spite of this the foxes continued to come in from the neighbouring country.

Trapping was started on 1st June, and was continued until 12th August. In all, 1,801 traps were set and 1,494 were sprung at 503 rabbit warrens. In the trapped area 43 sick and 75 dead rabbits were found during the priod 1st June to 16th August, when the last evidence of the presence of the disease was found. The sick rabbits were sometimes found 1 mile from the nearest trapped warren.

Observations were continued up to 3rd October, but they failed to reveal any appreciable effect on the density of the rabbit population in the trapped area. Breeding continued throughout, and the death rate from the disease, in so far as it may have reduced numbers, failed to balance the natural increase in the population.

5. General Conclusions.

1. The virus of myxomatosis is highly pathogenic and lethal for the European rabbit. It does not cause disease in other animals.

2. Myxomatosis spreads readily from rabbit to rabbit when close contact is provided. It does not spread readily if contact is not close and prolonged.

3. Rabbits kept in a relatively small enclosure are forced to maintain close contact with one another, and under these conditions a colony can be exterminated readily by the disease.

4. An epizootic of myxomatosis can be maintained for a long time in a colony in a small enclosure if fresh rabbits are introduced at short intervals and if other epizootic diseases are excluded.

5. The disease can be readily transmitted by species of *Echidnophaga,* the stick-fast flea, and by species of mosquitoes.

6. The disease can be readily transmitted also through injuries to the skin. Inoculation of rabbits has been successfully carried out by mechanical means with a modified rabbit trap.

7. Field experiments carried out over an area of 90 acres showed that when the disease was introduced into colonies of rabbits in the area it exterminated the rabbits in some of the colonies, but it did not spread from colony to colony or from warren to warren, and eventually died out.

8. A similar field experiment carried out on a rabbit population infested with the native stick-fast flea, *Echidnophaga,* showed that the disease spread from warren to warren and that a rabbit population of 500 was reduced to seventeen in a period of 60 days. As the flea has been shown to be a vector of the virus, it is assumed that the spread of the disease in this experiment was favoured by the presence of the insect vector.

9. Field trials, carried out over more extensive areas ranging from 300 acres to 7 square miles and under completely natural conditions including the presence of foxes and other predatory animals, gave results which showed that the method of distributing the disease with the aid of the trap can be successful in reducing a rabbit population under some conditions. Favourable results were obtained when foxes were not present in appreciable numbers, and especially when the rabbit population was infested with the native stick-fast flea. For the most part, however, the method failed to produce any appreciable effect on the rabbit population; the disease quickly died out. These unfavourable results seem to be due to the early removal of sick rabbits by foxes and other predatory animals before secondary and tertiary cases of disease could arise.

10. The general results of the study, and especially those of the field experiments and field trials, show that myxomatosis cannot be used to control rabbit populations under most natural conditions in Australia with any promise of success. Nevertheless, it seems possible that in some parts of Australia under special conditions, including the presence of insect vectors in abundance and the absence of predatory animals, the disease could be used with some promise of temporary control of a rabbit population. In any case, to be of any real value the disease would have to be used when the rabbit population density was moderate, not high, and a re-introduction would be necessary from time to time, probably annually, in order to keep the population density as low as possible.

Note.

The Director-General of Health granted permission for the virus to be brought into Australia for the experiments and field trials mentioned in this report. The virus has remained under the quarantine restrictions imposed by him. He has decided that it shall remain under his control, but the Council has undertaken to maintain it and not to supply it to any person or institution except with his approvel.

Transmission of Potato Virus Diseases.

4. Ground Work Studies on the Growth of Normal Potato Foliage.

*By J. G. Bald, M.Agr.Sc., Ph.D.**

Summary.

1. Periodical measurements of leaf area were made on potato plants of different varieties and strains during the earlier stages of development.

2. As in a previous experiment, the rate of growth of total leaf area was similar until about the inception of flowering: thereafter the growth rates of early strains and varieties were less than those of the later strains and varieties.

3. The early agreement in leaf growth rate indicated that the metabolic processes underlying growth at that stage were equally efficient in all strains and varieties tested. The numbers of leaves developing on the main axes were similar.

4. Differences in leaf area between strains and varieties seemed to arise from differences in the time of inception of several developmental stages.

5. In a variety, Up-to-Date, that has very large leaves on the main axis, the development of axillary shoots occurred later than in varieties with smaller leaves. Apparently continued expansion of the leaves on the main axis delayed the formation of axillary shoots.

6. The difference in the times at which axillary growth began was not evident between early and late strains of the same variety.

7. The main determinant of maturity was the growth rate of axillary shoots. Independent of varietal differences in the time of inception of axillary growth, early maturing strains and varieties had a relatively slow axillary growth rate, and late maturing strains and varieties had a relatively rapid axillary growth rate.

8. It is suggested that differences in maturity are mainly due to competition for growth-promoting substances between above-ground axillary shoots and those below ground on which tubers form (stolons).

1. Introduction.

Growth measurements of experimental potato plants have recently been needed as ancillary information in experiments on the transmission of potato virus diseases, and with the potato moth, *Gnorimoschema (Phthorimaea) operculella* (Zell.). Although many workers have studied the growth of the potato plant, and publications about it are numerous, the growth of the haulm has usually been described in biochemical or morphological terms having little application to these problems. The descriptions that have most nearly met the demands of our work are those of Werner (1934, 1940) and Stone (1933), but they have not always provided the kind of information needed. Growth studies, mainly on leaf area, were therefore begun, and a summary of the first results has already been published (Bald, 1943*a*). A further experiment on the same phase of growth has been made, and the results are presented in the present paper together with some more data from earlier studies.

* An officer of the Division of Plant Industry.

In the previous paper a description was given of a rating method for measuring the leaf area of relatively large numbers of potato plants growing in the field (Bald, 1943*a*). Its use was illustrated by data from an experimental area planted late in 1941 (1941-42, Plot 2) with early and main crop varieties. Allowing for differences between varieties in the dates of emergence, the growth rates of leaf area for all varieties were similar until early flowering. From then on the growth in leaf area of early varieties declined more rapidly than that of the later ones. As leaf areas in the early stages were similar, this difference implied that the later varieties attained a greater size of haulm than the early ones, a conclusion that agrees with general experience.

The initial coincidence and later divergence of the leaf-area growth rates in Plot 2, 1941-42, was retested early in 1943 by taking successive measurements of leaf area on individual plants during the earlier stages of growth. The 1943 experiment involved more detailed study of fewer plants. Its primary object was to discover how far sampling potato plants for aphids by the method of Davies (1934) was likely to give a true estimate of the total aphid population in a block of potato plants. It included four varieties. A preliminary phase in the study of the data was a comparison between the leaf area of the four varieties of which the plot was composed.

2. Description of Experimental Material.

(*i*) *Varieties Used.*

The varieties included in these experiments vary widely in habit of growth. Early Carman forms plants which Salaman (1926) would describe as of medium height, compact and vigorous. They do not usually grow beyond the stage at which the larger, earlier formed leaves mainly determine the character of the foliage. Late Carman is taller and the leaves appear somewhat smaller because of the relatively small leaves that develop during the later stages of growth. In contrast with Carman, Brownell is upright, open, and vigorous, with relatively long internodes at the base of the main stem. Snowflake, also, has a different habit. The stems are long and more prostrate, and the plant often has an open centre from which axillary shoots arise. The leaflets are narrow and widely spaced, and the leaves grow at an unusually acute angle from the stem. Up-to-Date and Delaware are somewhat like Carman, except that Up-to-Date is noted for its very large leaves.

(*ii*) *Description of the* 1941-42 *and* 1942-43 *Plots.*

Plot 2, 1941-42, which was described in a previous paper (Bald, 1943*a*), included the varieties Brownell, Early Carman, Delaware, and Up-to-Date planted in small frequently-replicated plots. The seed tubers were in excellent condition when they were planted, early growth was unchecked, and the plants were not pruned to two stalks as in 1943. They were better material than the plants of the 1943 experiment for the study of number and size of leaves on the main axis, particularly as they included the variety Up-to-Date which made the most interesting comparison with other varieties. Also there were small plots of Up-to-Date planted in other seasons which were closely comparable with Plot

2, 1941-42, in time of planting and seasonal conditions for growth. One of them (1942-43, Plot 1) grew in a clay loam about five miles from the site of the other plots, and was planted almost on the same day of the year as Plot 2, 1941-42. The other (Plot 1, 1941-42) was planted on October 13 in the same sandy loam as the Plot 2, 1941-42, and the plants emerged 3-4 weeks later than those of Plot 2 (Bald, 1943*b*).

Greater differences in date of planting than these have a considerable effect on the size of the leaves of potato plants, so, in this paper, for the study of leaf sizes attention is confined to plots growing in the early portion of the season. Intervarietal comparison can thus be made without confusion. The effects of growing the same varieties at different times of the year will be illustrated in another paper.

(*iii*) *Description of the* 1943 *Plot.*

The 1943 experiment included Brownell, Early Carman, Late Carman (a late strain of the same variety), and Snowflake, growing side by side in rows of 15 plants. In the same area as the 1943 plot, but not included in the experiment, were other varieties, among them Up-to-Date. Although the Up-to-Date seed tubers were badly shrunken when they were planted and the conditions for the growth of the plants were not entirely comparable with those of the varieties in the small experimental plot, measurements were also made on this variety. The measurements provided useful data for comparison with some of the results from the plots described in the last section.

The 1943 experimental area was planted on January 29. The seed tubers, which had been held over from the previous season, had long sprouts, but the sprouts were green and vigorous. The first records of emergence were made on February 8. Most of the plants that were studied in detail emerged and began to expand their first leaves between February 12 and 16. Development was exceptionally rapid because of warm temperatures and diminishing day length conditions.

During most of the period of growth rain was insufficient to supply the water requirements of the plot. It was irrigated twice, once on February 24 after the plants had begun to wilt during the heat of the day, and again on March 12. The effect of water shortage between February 19 and 23 was shown by small increments in leaf area (Table 1), but during the period February 24 to 26 the increments were correspondingly great. The leaves that had already developed expanded very rapidly, and appeared to make up almost completely the deficiency of growth due to the temporary drought. This sort of reaction is characteristic of processes governed by more than one limiting factor and is comparable with the effects of nitrogen administered to nitrogen-deficient potatoes (Werner, 1934).

Cultivation of the plot was sufficient to prevent the growth of weeds and the surface soil from caking. The plants, which were planted sufficiently wide apart to prevent competition between them, were not hilled in case the lower leaves should be covered with soil or injured. Hence, dying of the small bottom leaves, which occurred in many instances, was not due to injury by burial or cultivation.

The earliest maturing plants were dug during the last two weeks of April after the tops had died. These included the Early Carmans, and four Brownells, which were found to belong to an earlier maturing strain than most other plants of this variety.

The remaining plants of the late varieties were killed by frost on May 12, and were dug some time later. At digging it was noticed that the two stalks of some plants, particularly amongst the Snowflakes, were axillaries originating underground from a main shoot, the tip of which had been injured and had failed to grow. On other plants the shoots were separate and had independent root systems; and the stalks of others were too decayed to distinguish to which type they belonged. Where the stalks were known or suspected to arise from the same sprout the pair of stalks was treated for purposes of calculation as a single shoot.

The reason for this was that together they behaved like a single main shoot. There was evidence of competition between the secondary axillaries on the two primaries as there is between axillaries on the same main shoot. Therefore, the total areas of leaves arising directly from the two primary shoots were combined and taken as the leaf area of the main shoot; and the leaf area of all axillaries arising from them was taken as the axillary leaf area.

(iv) Method of Measurement.

In the 1941-42 and 1942-43 plots, very few measurements were made successively on the same plants, as only standards for rating whole plants were measured, and they were chosen on each occasion when measurements were made, almost at random in the experimental areas. Every leaf of a plant was measured by the leaf rating method (Bald, 1943*a*; Thirumalachary, 1940), and measurements of leaves on the main and axillary shoots were kept distinct. These data have been used mainly in the study of the average size of leaves on the main shoots of different varieties.

In the 1943 experiment, also, the leaf rating method was used. Records were kept so that the increase in size of each leaf could be followed throughout the course of the experiment, and measurements of leaves on main and axillary shoots were kept distinct. Bi-weekly and, later, weekly measurements were made of all leaves on six plants of each variety. These six were chosen for measurement from fifteen plants that were originally planted. The criteria for the choice were that they emerged within the same period of about ten days, and that they had healthy and reasonably vigorous shoots. All main shoots but the first two to emerge were pruned off at ground level. The six plants of each variety were measured during the whole course of the experiment, but from amongst them two were chosen for intervarietal comparison. These in turn were chosen because they emerged during the same half-weekly period, and had two main shoots of approximately equal vigour and leaf area at the first two half-weekly measurements. The results from the other four plants of each variety have been used as a check on the conclusions arrived at from the two finally selected plants, but in most instances these data are not presented in detail.

One Snowflake plant (3-9, Table 1) of the pair finally chosen was later found to be infected with *Fusarium* wilt. Its growth rate was reduced by infection, so data from it were not included in the calculations; instead the data for the two plants in Table 2 were substituted.

TABLE 1.—LEAF AREA IN SQ. CM. OF PLANTS OF 4 VARIETIES. AREAS OF MAIN SHOOTS (M), AXILLARY SHOOTS (A), AND THE SUM OF THE TWO (T).

Variety.	Plant.	Shoot.	Date— February— 16th.	19th.	23rd.	26th.	March— 2nd.	9th.	17th.	18th.	24th.	25th.
Early Carman	1–6	1 M	92	194	250	442	554	1,030	1,121	..	1,162	..
		A	..	..	..	50	140	341	693	..	789	..
		T	92	194	250	492	694	1,371	1,814	..	1,951	..
		2 M	60	165	285	499	788	1,303	1,076	..	2,009	..
		A	..	..	..	60	110	196	389	..	553	..
		T	60	165	285	559	898	1,499	2,365	..	2,562	..
	1–15	1 M	91	212	253	583	848	857	755	..	900	..
		A	..	..	20	100	170	420	698	..	772	..
		T	91	212	273	683	1,018	1,277	1,453	..	1,672	..
		2 M	102	186	313	419	615	920	1,141	..	1,196	..
		A	..	..	10	70	141	256	364	..	504	..
		T	102	186	323	489	756	1,176	1,505	..	1,700	..
Late Carman	2–4*	1 M	60	255	435	643	957	905	930	..	930	..
		A	..	..	50	181	562	1,624	2,826	..	3,284	..
		T	60	255	485	824	1,519	2,529	3,756	..	4,214	..
		2 M	50	171	279	538	853	1,171	1,410	..	1,631	..
		A	..	..	..	50	141	302	780	..	1,213	..
		T	50	171	279	588	994	1,473	2,190	..	2,844	..
	2–9	1 M	119	261	401	532	790	913	897	..	937	..
		A	..	..	..	60	182	504	882	..	1,376	..
		T	119	261	401	592	972	1,417	1,779	..	2,313	..
		2 M	40	60	141	244	372	652	1,014	..	1,074	..
		A	..	..	..	..	50	132	353	..	923	..
		T	40	60	141	244	422	784	1,367	..	1,997	..
Snowflake ..	3–9†	1 M	71	126	175	303	380	526	605	..	773	..
		A	..	..	10	50	80	191	335	..	474	..
		T	71	126	185	353	460	717	940	..	1,247	..
		2 M	71	150	201	336	423	566	739	..	842	..
		A	..	..	..	30	70	182	413	..	749	..
		T	71	150	201	366	493	748	1,152	..	1,591	..
	3–14*	1 M	120	213	281	513	874	1,227	1,581	..	1,748	..
		A	..	..	30	101	245	553	1,702	..	3,043	..
		T	120	213	311	614	1,119	1,780	3,283	..	4,791	..
		2 M	50	147	238	398	564	1,103	1,230	..	1,367	..
		A	..	..	..	..	40	101	316	..	1,759	..
		T	50	147	238	398	604	1,204	1,546	..	3,126	..
Brownell ..	4–1	1 M	72	276	490	731	1,030	1,360	..	1,619	..	1,793
		A	..	..	30	100	150	330	..	541	..	987
		T	72	276	520	831	1,180	1,690	..	2,160	..	2,780
		2 M	30	115	244	528	935	1,269	..	1,551	..	1,544
		A	..	..	..	60	110	337	..	722	..	1,071
		T	30	115	244	588	1,045	1,606	..	2,273	..	2,615
	4–14*	1 M	121	233	432	704	1,048	1,318	..	1,626	..	1,733
		A	..	..	..	110	262	1,039	..	1,890	..	3,231
		T	121	233	432	814	1,310	2,357	..	3,516	..	4,964
		2 M	78	203	319	573	867	1,042	..	1,350	..	1,490
		A	..	..	..	60	90	487	..	1,050	..	1,449
		T	78	203	319	633	957	1,529	..	2,400	..	2,939

* These plants were known or suspected to consist of two axillary shoots arising underground from a main shoot the tip of which had been injured and failed to grow. For purposes of calculation, the figures for the two shoots were bulked and treated like one shoot.

† This plant was infected during its growth with *Fusarium* wilt, and the data have been excluded from the general calculations. The two plants for which data are given in Table 2 were substituted.

TABLE 2.—LEAF AREA IN SQ. CM. OF TWO SNOWFLAKE PLANTS, MAIN SHOOTS (M), AXILLARY SHOOTS (A), AND TOTAL (T).

Plant.	Shoot.	February—					March—			
		12th.	16th.	19th.	23rd.	26th.	2nd.	9th.	17th.	24th.
3–3	M	91	351	525	685	1,182	2,019	2,450	2,628	2,628
	A	..	..	..	100	252	675	1,690	3,901	5,838
	T	91	351	525	785	1,434	2,694	4,140	6,529	8,466
3–4	M	..	..	166	349	834	1,284	2,134	2,906	3,231
	A	..	..	..	..	50	249	784	2,331	3,939
	T	..	..	166	349	884	1,533	2,918	5,237	7,170

The careful selection of plants simplified examination of the results, and introduced no observable bias into the conclusions. Nearly all the plants for intervarietal comparison began to open their first flowers on the cymes of the main stems about March 9, little more than three weeks after the shoots emerged from the ground. Measurements were continued for another two weeks. During those two weeks infestation by the potato moth seriously damaged the foliage. It was possible for a short period to measure and compensate for the destruction of leaf tissue with a fair degree of accuracy, but thereafter the accumulated effects of the loss of tissue made such corrections inaccurate and of little value, and the measurements were discontinued. Hence the experiment gives information only about the period before the major portion of the plants' metabolic energy was diverted from the formation of foliage and roots to the development of tubers. The figures given for the final two weekly measurements have been corrected for moth damage; the earlier figures are actual measurements.

In tabulating the results of the 1943 experiments, values of leaf area for each main shoot and the total for the axillary shoots arising from it were kept separate. Where the two shoots of one plant were known or suspected to arise underground as axillaries from the same main shoot (see last section), the total areas of leaves arising directly from the two primary shoots were combined and taken as the leaf area of the main shoot; and the leaf area of all axillaries arising from them was taken as the axillary leaf area.

The results of this experiment are used for a closer analysis of the increase in leaf area than was possible with the bulk totals from Plot 2, 1941-42. On the other hand, the number of plants involved is small, and natural variations in size from plant to plant cause wide variations in the leaf area totals. Direct comparison of leaf areas between varieties during any particular period of time is therefore of little value as an indication of differences in growth (e.g., see Table 1). The data for most purposes are put in the form of logarithms of the actual values, and growth rates are used as the criteria of agreement or divergence between varieties. In this way the data are examined for what they reveal of the development of main and axillary shoots.

3. Increase in Total Leaf Area, 1943. Experiment.

Included in Tables 1 and 2 is the total leaf area in sq. cm. of nine plants selected for inter-varietal comparison. A preliminary examination revealed no systematic differences in the growth rate between varieties, until the initiation of flowering. There was as great a variation between plants of the same variety as between varieties. After the initiation of flowering the later strains and varieties grew more rapidly than the earlier, as previously reported (Bald, 1943*a*). These facts are not immediately apparent from the arithmetic totals in Tables 1 and 2, but they appear when the figures are converted to logarithms and plotted, particularly if mean values for all the shoots of each variety are used for the comparison.

4. Development of the Main Axis, Number and Size of Leaves.

From the literature on the growth of the potato it appears that the foliar portion of a normal potato plant has a definite cycle of development, depending on the regular development of individual leaves, and therefore probably has a definite form. It is known that successive leaves on the main shoot go through similar cycles of growth (Stone, 1933). Also, "apparently elongation in the potato plant is genetically limited to a fairly definite number of nodes" (Werner, 1934). Such facts are bound up with the development of total leaf area, and may be used in the search for an explanation of agreement or divergence in leaf area between different strains and varieties. It will be shown that the limiting number of leaves on the main axes is similar for the varieties examined, and that, where differences in leaf area of the main axes exist, they must be due to differences in the average *size* of leaves.

For the main shoot Werner (1934) gives the following figures for leaf number: fifteen to nineteen leaves with axillary buds above ground, and ten to fourteen nodes from which stolons may develop below ground. Similar counts were obtained at Canberra during two seasons with a number of varieties. Although, below ground, the numbers of nodes counted were mostly between eight and ten, they were often difficult to assess, and our figures may be too low. No consistent difference in the number of leaves was observed between varieties. The size of sprouts on the seed tubers before planting seemed to affect the number of leaves on the main stem more than did varietal differences. If the seed tubers carried relatively long green sprouts with many nodes, the number of nodes above ground on shoots growing from them was less than normal—as low as thirteen. The number was occasionally further reduced if the tip of the sprout was injured at planting, and growth was accomplished by axillary shoots arising below ground level. However, these variations did not noticeably affect the rates of growth in leaf area.

The commonest numbers of nodes above ground on the main stems of well-grown plants are seventeen and eighteen. Of these generally one or two nodes at the base of the stem produce small leaves that die off early or they may produce only leaf rudiments. In the mature shoot the commonest numbers of leaves actually present on the main axes are therefore fifteen to seventeen.

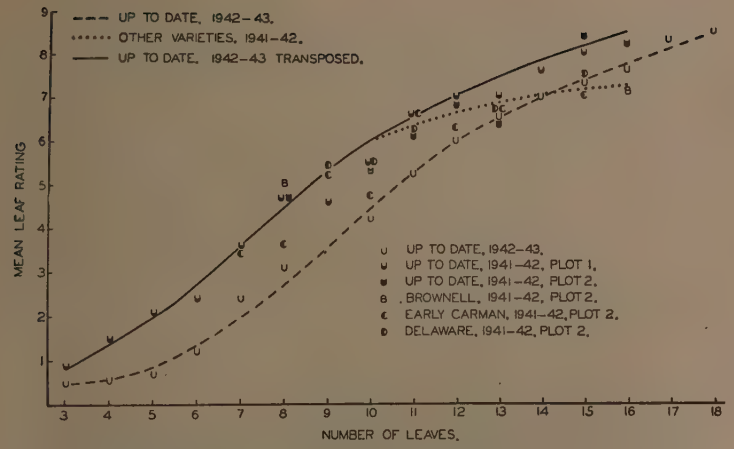

FIG. 1.—Mean size of leaves on the main stalks of potatoes, given in the form of ratings for area: data from three experiments in two seasons.

TABLE 3.—MEAN LEAF RATINGS OF MAIN SHOOTS BEARING DIFFERENT NUMBERS OF LEAVES. DATA FOR FOUR VARIETIES GROWN DURING EARLY SUMMER.

Variety.	Number of Leaves—													
	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
Up-to-Date 1942–43 ..	·5	·6	·7	1·2	2·4 3·1	3·4	4·2 5·2	6·0	6·4	7·0	7·3	7·6	8·3	8·5
Up-to-Date 1941–42 (Plot 1) ..	·9	1·5	2·1	2·4	3·6	4·7	4·6	5·5	6·6	7·0	7·0	7·6	8·0	..
Up-to-Date 1941–42 (Plot 2) ..	..	..	..	..	..	4·7	..	5·3	6·1	6·8	6·4	..	8·4	8·2
Brownell 1941–42 (Plot 2) ..	..	..	..	..	..	5·0	..	..	..	..	..	..	..	7·1
E. Carman 1941–42 (Plot 2) ..	..	..	..	..	3·4	3·6	5·2	4·6	6·6	6·3	6·7	..	7·0	..
Delaware .. 1941–42 (Plot 2) ..	..	..	..	..	..	..	5·4	5·5	6·2	..	6·7	..	7·5	..

As the number of leaves on the main axis is nearly always close to a constant value, the progressive stages in the development of a shoot may be defined by the number of leaves that have expanded below the terminal whorl. The mean of the areas for single leaves then becomes a measure of the leaf area of the central shoot, and offers a useful method of comparison between varieties, and between different lots of the same variety. Data of this kind are presented in Tables 3 and Fig. 1. The results are given as mean leaf-area ratings. As the ratings are made on a logarithmic scale (Bald 1943*a*), these are equivalent to geometric means.

The first set of means in Table 3 is derived from the schema for the growth of a single Up-to-Date shoot shown in Table 4. This in turn is derived from series of leaf area measurements made on plants used as rating standards in the 1942-43 plot. Ratings of leaves in the same relative positions on shoots carrying the same numbers of leaves were averaged; these mean figures are given with slight alterations in Table 4. The alterations were well within the scale of variability of the original data and were made to eliminate small inconsistencies, so that the schema might suggest the serial development of individual leaves on one typical shoot. The period covered is from one or two days after the shoot emerged until the initation of flowering. By the end of this period axillary shoots were growing from the lower nodes and had just begun to grow from the second or third nodes below the flower head.

TABLE 4.—PLAN OF GROWTH IN LEAF AREA OF A SINGLE UP-TO-DATE SHOOT FROM EMERGENCE UNTIL THE INCEPTION OF FLOWERING. DERIVED FROM AVERAGES FOR PLANTS IN LEAF ROLL TRANSMISSION EXPERIMENT, DICKSON, 1942-43, AVERAGE FIGURE SLIGHTLY MODIFIED TO GIVE SMOOTH SERIES OF VALUES. GROWING TIP (T) REGARDED AS NON-FUNCTIONAL AND NOT INCLUDED IN SUMS OF RATINGS OR MEANS.

Leaf Ratings.	Sum of Ratings.*	No. of Leaves	Mean Rating.
0 0 0 T	1·5	3	·5
0 0 1 0 T	2·5	4	·6
0 0 1 1 0 T	3·5	5	·7
0 0 1 2 2 1 T	7	6	1·2
0 0 2 4 5 4 1 T	17	7	2·4
.. 0 2 4 6 5 3 1 T	21·5	7	3·1
.. 0 2 3 6 6 5 3 1 T	27·5	8	3·4
.. 0 2 4 7 8 7 5 3 1 T	37·5	9	4·2
.. .. 3 5 7 8 8 7 5 3 1 T ..	47·0	9	5·2
.. .. 3 5 8 9 9 8 7 6 4 1 T ..	60·0	10	6·0
.. .. 3 5 8 9 9 9 8 8 6 4 1 T ..	70	11	6·4
.. .. 3 6 9 10 10 10 9 9 8 6 3 1 T ..	84	12	7·0
.. .. 3 6 9 10 10 11 10 10 9 7 6 3 1 T ..	95	13	7·3
.. .. 3 6 9 10 11 11 11 11 10 8 7 6 3 1 T ..	107	14	7·6
.. .. 2 6 9 10 11 12 12 12 11 11 10 8 6 3 1 T ..	124	15	8·3
.. .. 2 5 9 10 11 12 12 12 11 11 10 9 8 7 5 1 F	135	16	8·5

* Rating of 0 given a value of ·5.

Rating Scale for Leaves	Rating..	0	1	2	3	4	5	6	7	8	9	10	11	12
	Leaf area (sq. cm.)	10	21	27	34	43	55	70	89	114	145	184	234	299

The mean ratings are plotted in Fig. 1, with others from Table 3, against the numbers of leaves present on the shoot. In the table, the 1942-43 figures are given according to the number of leaves actually present at each stage, and there are therefore two figures in each of the columns for seven and nine leaf means. In plotting the data the number of leaves is taken as the total number formed, and no account is taken of the disappearance of the basal two. Two other sets of data for the same variety, collected during the previous season, 1941-42, are also set out in Table 3 and Fig. 1. These are derived from plots described in the introductory portion of the paper. Each figure is a mean rating derived from 2-8 shoots having an equal number of leaves.

A smooth curve (broken line) was drawn through the points for Up-to-Date 1942-43. This curve was then transposed on the assumption that, on most of the 1941-42 plants, the two small basal leaves were not formed or were too rudimentary for measurement. An examination of the primary data suggested this as an explanation of the higher values for equivalent numbers of leaves revealed in Table 4. The transposed curve was found to fit closely the majority of values for Up-to-Date 1941-42, i.e., in two seasons the mean size of leaves on main stalks at the same stage of development were closely similar.

The other varieties in 1941-42, Plot 2, were also similar to Up-to-Date until eleven or twelve leaves were formed, but by the time the shoot had developed fifteen or sixteen leaves, the mean ratings of Brownell, Early Carman, and Delaware taken together were significantly less than Up-to-Date.

At first sight it appears from this analysis that differences in size of leaves on main shoots of Up-to-Date and the early varieties may partly account for the differences in leaf area that develop in the later stages of growth. On the other hand, the difference in leaf size developed *before* differences in total leaf area became apparent. Also, the total leaf area of Brownell, which has leaves similar in size to those of the early varieties, Early Carman and Delaware, follows practically the same curve of increase as Up-to-Date (Bald, 1943*a*). This discrepancy may be resolved by examining the time of inception of axillary growth and the leaf area of main and axillary shoots.

5. Leaf Area of Main and Axillary Shoots.

From this point the nature of the data makes it inconvenient to follow a logical order in discussing the inception and growth of axillary shoots. To put the discussion on the leaf area of main and axillary shoots in perspective, results of data presented in the next section will be briefly summarized. The larger size finally attained by leaves of Up-to-Date plants is associated with some delay in the inception of axillary growth. The energy that in varieties such as Brownell and Carman is expended in the formation of new leaves on axillary shoots is for a time absorbed by Up-to-Date plants in increasing the size of leaves already formed on the main shoot. This explains why the growth rates for total leaf area may not exhibit differences between some varieties at this stage when it is obvious that there are differences in the average size of leaves.

The varieties used in the 1943 experiment (leaving out of account the nearby Up-to-Dates) have leaves of similar size on the main stem, although the form of the leaves is distinct and characteristic of the variety.

During the period when growth rates of the different varieties were similar, the bulk of the leaf area was made up of leaves on the main stalks. Axillary shoots began to reach measurable size on February 23 or 26, less than a fortnight after the emergence of the main shoots. By the time the last measurements were made they constituted a large part of the leaf area, in some instances more than half. The size of axillary shoots seemed largely to determine varietal differences in leaf area during the stage of growth after the initation of flowering.

The leaf areas of the main and axillary shoots were therefore calculated separately. The data for the two shoots of each plant, or for the whole plant where the two shoots arose from the one axis, were converted to logs and plotted against the dates of measurement. There was some variation in the form of curves for single shoots, but there were no consistent differences between varieties in the rate of development of the *main* shoots until the time the last reading was taken. The final leaf area of *axillary* shoots was greater for the later varieties than for the Early Carman. The difference was accounted for rather by a greater rate of growth than by differences in the time of appearance of the axillary shoots, as was proved by comparing the average daily rates of growth in leaf area of the axillary shoots.

The main difficulty in making this comparison was to find a common reference point on the time scale. In spite of care in the original choice of plants, in some instances shoots even on the same plant were not simultaneously at the same stage of development. The date of emergence for each shoot was not accurately known, as observations were made at three or four day intervals. An objective reference point was obtained by interpolation from the graph for log total leaf area of each shoot and the log leaf area of its axillary shoots. The reference point was taken as the date on which the area of the axillary shoots was one eighth the total leaf area (= a difference of ·9 on the ordinate). The increase in log leaf area from this date until the last measurement was divided by the number of days to give the mean daily growth rate. The results are given in Table 5.

Table 5.—Data used in Calculating Growth Rates for the Leaf Area of Axillary Shoots. Zero Time was Taken as the Day on which the Axillary Leaf Area for each Shoot was One-Eighth the Total Leaf Area of the Shoot.

Variety.	Plant.	Shoot.	Zero Time.	Log Zero Leaf Area.	Log Final Leaf Area.	Diff.	Time (days).	Growth Rate.
Early Carman	1–6	1	27/2	1·82	2·90	1·08	25	·043
	..	2	2/3	2·04	2·74	·70	22	·032
	1–15	1	25/2	1·78	2·89	1·11	27	·041
	..	2	26/2	1·85	2·71	·86	26	·033
Late Carman	2–4	1 + 2	25/2	2·16	3·65	1·49	27	·055
	2–9	1	27/2	1·90	3·14	1·24	25	·050
	..	2	2/3	1·70	2·97	1·27	22	·057
Snowflake	3–3	1 + 2	23/2	2·00	3·77	1·77	29	·061
	3–4	1 + 2	1/3	2·22	3·60	1·38	23	·060
	3–14	1 + 2	28/2	2·22	3·68	1·46	24	·061
Brownell Early ..	4–1	1	26/2	2·00	2·99	·99	27	·037
	..	2	28/2	1·94	3·03	1·09	25	·044
Brownell Late	4–14	1 + 2	27/2	2·31	3·67	1·36	26	·052

The axillary growth rates are reasonably consistent and correspond with the order of maturity of the different strains and varieties. The Early Carmans matured during the last two weeks of April (mean growth rate ·037), the early Brownell plant had matured by May 1 (·041), Late Carmans and Brownell were still green on May 12, when they were killed by frost, but they normally mature at about the same time (·053 and ·052), and Snowflake is the latest of the commercial varieties commonly grown in Australia (·061). Analysis of the figures in the last column of Table 5 showed that differences between the early and late strains as a whole were highly significant and the difference between Late Carman and Brownell combined and Snowflake was nearly significant at the 5 per cent. level of probability.

The agreement of growth rates for main shoots and the divergence for axillary shoots is illustrated in Fig. 2, where all the data for the nine plants of the different varieties are plotted. To obtain this figure the data for each shoot or plant were shifted on the time scale (abscissa) according to the number of days before or after February 27 that the axillary leaf area reached the level of one-eighth the total leaf area. This gave a uniform time scale applicable to all shoots. The curves for the main shoots were obviously of a similar form but did not coincide, because some shoots or plants were larger than others throughout the whole period of the experiment. By shifting each curve as a whole up or down the scale of leaf area (ordinate) they were all revealed as of essentially the *same* form.

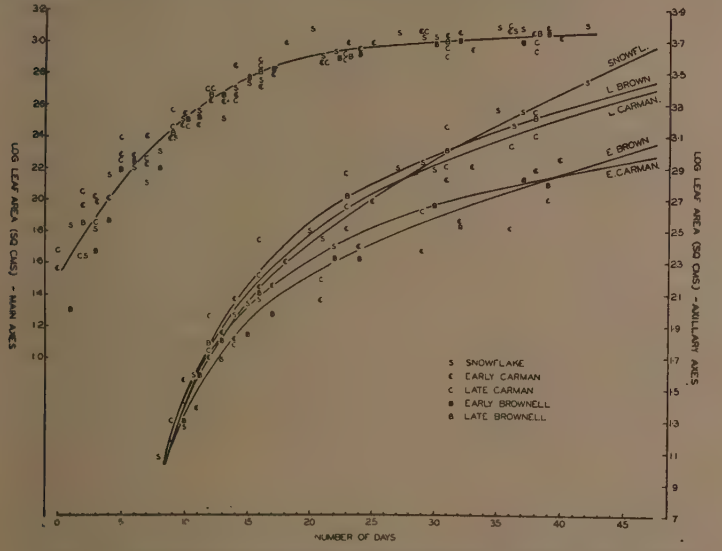

FIG. 2.—Growth curves for main shoots (above) and axillary shoots (below) of five early and late strains or varieties of potato, 1943.

The shifting of the curves was made independent of individual judgment by adopting the following method. Log leaf area for the first main shoot of Early Carman (1-6, Table 1) was plotted against time, and the points were joined by straight lines. A value for log leaf area on each day during the course of growth was read off from the graph and inserted in a table of log leaf area for

all plants, tabulated according to the adjusted time scale. For each shoot the average difference was found between the log of the readings for leaf area and the corresponding value, real or interpolated, for shoot 1 of Early Carman 1-6. The values for the first nine days on the adjusted time scale were excluded from the averages, because the plants were then small, and measurement was relatively inaccurate. Also, additional variability had been introduced by the drying out of the soil (p. 96).

The mean differences were added to or subtracted from the log of all the recorded values for both the main and axillary shoots. Because of the conversion of the data to logs, this is equivalent to multiplying all values of leaf area for a main shoot and for its axillaries by a constant figure, smaller or greater than unity, derived only from the data for the main shoot. Thus an unbiased comparison may be made between the adjusted values for axillaries on different shoots.

The curves for axillary shoots show a divergence in growth rates almost from the first. The axillary shoots on the late varieties attain their greater size by growing faster than the axillaries on the early varieties rather than by beginning growth earlier. This is well illustrated by Early and Late Carman. The plants for which data are given in Table 1 emerged within a day or two of each other. The axillary shoots emerged about the same time. When they comprised one-eighth the total leaf area (24-25 days before the final measurement) their leaf area was on the average 0·75 sq. dm. per main shoot. The final leaf area of the axillary shoots was 6·46 sq. dm. for Early Carman and 15·05 sq. dm. for the Late Carman. At this time the areas for main shoots were Early Carman 13·60 sq. dm., Late Carman 10·35 sq. dm. These are geometric means derived from the unadjusted values, and the data for the two shoots of Late Carman 2–4, which probably arose from the same main axis, were not bulked on this occasion.

The development of the leaf area on upper and lower groups of axillary shoots was examined to see if the lower growth rate of early strains was reflected more in one group than in the other.

On all varieties the first axillary shoots to develop are towards the base of the main stem, but, later, axillary shoots develop from the nodes below the terminal flower bunch. Generally the shoot from the second or third node below the flower head becomes dominant, but one or two other axillary shoots both above and below it grow to measurable size. From the nodes between the upper and lower groups there is only feeble development of axillary shoots or none at all. There was no indication that either group of axillaries was mainly responsible for differences in axillary growth rates: the upper and lower groups of the earlier strains and varieties both grew more slowly than the corresponding groups of the later strains and varieties.

The axillary shoots can therefore be regarded as a unit section of the plant because they are subjected to similar influences. The nature of these influences is suggested by the early development of stolons and tuber initials on early strains and varieties (Werner, 1940). Competition exists between the axillary shoots and stolons. If the development of the vegetative and storage organs is controlled by growth-promoting substances, it seems probable that a partition of these substances between above-ground axillary shoots and below-ground

axillary shoots (stolons) must occur. Genetical control in turn presumably determines the balance of the partition, and the relative growth of axillary shoots and stolons.

Summarizing, there is reasonable agreement between early and late varieties, until the initiation of flowering, in the growth rates of the foliage as a whole. The general agreement, which was demonstrated both in a previous paper (Bald, 1943*a*) and in this one, hides an important difference in the rate of axillary growth. This becomes obvious only at about the inception of flowering, when the axillary shoots have become a quantitatively important element of the foliage. Until then, agreement in the foliar growth rates of the main stems overwhelms the small difference introduced by disagreements in the growth rates of axillary shoots.

6. Time of Inception of Axillary Growth.

It has already been shown that Up-to-Date produces larger leaves on the main stem than other varieties grown under similar conditions, and it has been suggested that this is due to continued expansion of these leaves and a corresponding delay in the inception of axillary growth. It appears from Fig. 2 that the varieties Early and Late Carman, Snowflake, and Brownell which bear on the main stem leaves of about the same average size, begin axillary growth at the same time. It remains to show for Up-to-Date that, in association with the development of larger leaves on the main stem, there is a delay in the inception of axillary growth.

Two kinds of evidence were studied. First a comparison was made with late Carman of the proportion of the total leaf area carried by axillary shoots on March 9. Late Carman and Up-to-Date are similar in maturity, and the six plants of each that were measured emerged on the average about the same time. All six plants of each variety were included in the comparison. The leaf area of axillary shoots on the Late Carman plants comprised 45 per cent. of the total leaf area, and on Up-to-Date only 23 per cent. On one Up-to-Date plant, which was comparable in vigour and date of emergence with the two Carmans selected for intervarietal comparison, the axillary leaf area was 19 per cent. of the total. The axillary growth rate for the 23 days from March 2, when the axillary leaf area was one-eighth the total leaf area, until March 25, was ·052. This agrees well with ·053 for Late Carman and ·052 for Late Brownell, which are comparable in maturity to this strain of Up-to-Date.

The other form of evidence was more direct. The closest possible estimate of the date of emergence* was made for each shoot of the nine plants for which figures are given in Tables 1 and 2, and for the two shoots of the one comparable Up-to-Date plant. The number of days between emergence and the date when the axillary leaf area was one-eighth the total leaf area was then found.

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* The date when the axillary leaf area was one-eighth the total leaf area has so far been used as a reference point on the time scale because it could be calculated with more precision than the date of emergence.

The mean figures and the standard deviation of a single observation are given in Table 6.

Table 6.—Mean Values for Number of Days Between the Emergence of Main Shoots from the Soil, and the Date at which the Leaf Area of Axillary Shoots was Equal to one-Eighth the Total Leaf Area, 1943 Experiment.

	Early Carman.	Late Carman.	Snowflake.	Brownell.	Up-to-Date.
Number of days ..	12·75	12·67	13·0	13·0	16·0
Replicates	4	3	3	3	2

Standard deviation = 1·16.

The difference between Up-to-Date and the four other varieties combined is significant. On the one Up-to-Date plant, the inception of the growth of the axillary shoots probably occurred about three days later than on plants of the other varieties. For other Up-to-Date plants there was evidence of a similar delay.

7. Discussion.

The following discussion applies only to the first portion of the potato plant's life-history. It contains no suggestion of what may happen to the foliar portion of the plant during the periods of maturity and senescence. Also, it is possible that the schema devised to co-ordinate the experimental facts may over-simplify the pattern of development, and it is therefore put forward only as a first approximation to the truth.

The pattern of growth in leaf area postulated in this paper is shown in diagrammatic form in Fig. 3. Along the abscissa is the time scale which under the conditions of the 1943 experiment would represent 40 days from emergence. Early in the season growth to an equivalent stage of development would occupy about 60 days.

The various curves for leaf number, leaf size, and leaf area are plotted on the same time scale. The broken lines represent a variety with large leaves and the plain line a variety with smaller leaves. In plotting axillary growth, curves for early and late strains of each variety are drawn. In drawing the curves for leaf size, and total leaf area of the main shoots, it is assumed that values for early and late strains coincide. For the curve of leaf number it is assumed that values for both strains and varieties coincide. Also the number of leaves includes the basal nodes at which very small leaves may not be produced or may be produced and die prematurely. The value plotted is the number that have expanded below the terminal whorl.

According to the time scale in Fig. 3, all leaves on the main shoot have expanded by the twentieth day after emergence and the flower buds begin to open about the twenty-third day. During the first ten days after emergence, eleven leaves are formed without any distinction in mean size between large-leaf and small-leaf varieties. By the tenth day also the small-leaf variety has begun to produce leaves of measurable

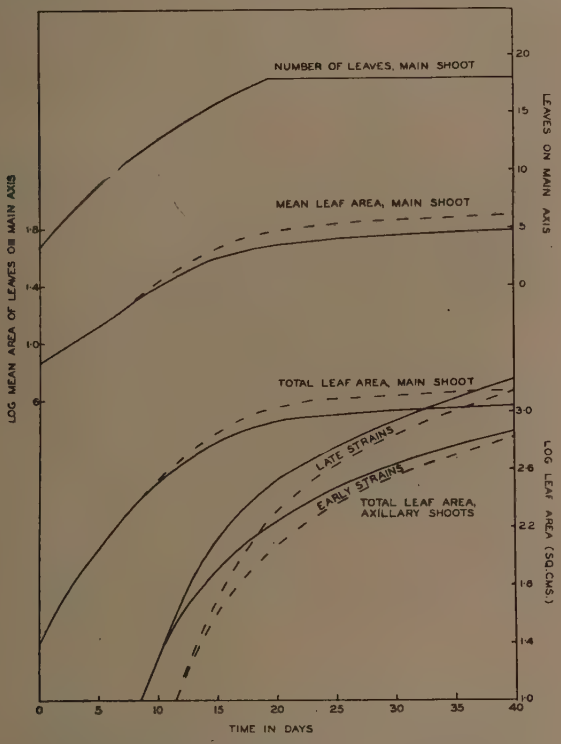

FIG. 3.—Diagrammatic illustration of the development of the leaves on shoots of early and late strains of two varieties of potato, one with large leaves (broken line) and one with smaller leaves (plain line). The number of leaves is taken as the number expanded below the terminal whorl, and includes the basal nodes on which only rudimentary leaves may develop. The time scale is short because it represents a period under the diminishing day-length conditions of autumn. Leaf areas are plotted on a logarithmic scale.

size from axillary shoots, whereas the large-leaf variety fails to do so until about the thirteenth day. The rate of axillary growth for early and late strains is distinct, but the axillary growth rates of strains equivalent in maturity, whether they belong to the large-leaf or the small-leaf type, are similar, in spite of the different times at which their growth begins. How far this correspondence is likely to persist after the stage of development depicted in Fig. 3 it is impossible to say.

Of the several features of this plan of development, that which is most securely established is the association between the growth of axillary shoots and maturity*. It is confirmed by observations and measurements other than those already cited. Of the observations one instance may be given. A few Early and Late Carmans were grown in pots in a quarantine insectary at Canberra under conditions of very low light intensity. At maturity they were etiolated and the normal flower heads were replaced by almost microscopic rudiments, but the numbers of leaves on the main stems were the same as in the field, and the growth of axillary shoots was obviously less on the Early Carman than on the Late Carman.

The results of an additional set of measurements are worth recording. Amongst the Snowflakes in the plot which provided most of the data for this paper was one plant that had ripened off by May 10, two days before the late varieties were killed by frost. Its intermediate maturity was reflected in its axillary growth rate ·044, which lay between the values for early and late strains and varieties (see Table 3). The growth of the main shoot followed the same course as those of the other Snowflakes. The measurements from this plant have not been given as a third example of an earlier strain, having a slower axillary growth rate than later strains of the same variety, because the tubers were found to have purple eyes, and the tubers of Snowflake are normally free from colour around the eyes. Although it was probably of a different variety, the data obtained from it fit in well with those given earlier.

As stated in the introduction, the objects of the leaf measurements made on potato plants were to discover how growth affected sampling for aphids and the estimation of damage by the potato moth *G. operculella*. These aspects of the investigation will be reported elsewhere. The results presented here have a wider application. They supplement Werner's work (1934, 1940) on the physiology of early and late-maturing strains, and they suggest a more rational outlook on certain phases of breeding for foliage type, maturity, and yield in potatoes. Some components of these characters appear to be variable and some relatively invariable. This suggestion should at least be of negative value to plant breeders. For example, if the pattern of development described here is correct, there appears little chance of obtaining from ordinary commercial material a variety that matures quickly because it develops fewer nodes on the main stalk; whereas a study of leaf size, time of inception of axillary shoots, and growth rate of axillary shoots, in parent varieties or seedlings, might help materially in the process of breeding early or late-maturing varieties.

8. Acknowledgment.

The author wishes to thank Mr. G. A. McIntyre for advice on statistical matters.

* Amongst a group of modern American varieties, of which Katahdin is the best known, the balance of growth between main stems, axillaries, and tubers may not be quite the same as in the varieties described here. However, the same series of developmental stages probably exists, and the later varieties appear to have a higher rate of axillary growth than the earlier varieties.

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Note on the Estimation of the Effect of Diurnal Temperature Fluctuations on Reaction Rates in Stored Foodstuffs and Other Materials.

*By E. W. Hicks, B.A., B.Sc., A.A.C.I.**

Summary.

Since the relation between the rate of the "ageing" reactions in foodstuffs and temperature is exponential and not linear, the effective mean temperature appropriate for calculating the extent of these reactions is higher than the arithmetic mean. Formulae are developed for the estimation of the difference between these two means, and a table of values is given. It is concluded that for reactions of Q_{10} value of the order of 2, this difference is of little practical importance for storage where there is little direct effect of solar radiation, but it may be large for goods exposed to the sun.

In food preservation investigations, storage trials at a series of constant temperatures are often carried out, and it is frequently necessary to estimate from the results of such trials the storage life of a commodity held at ordinary temperatures in a particular locality. It is usual to base such calculations on a temperature estimate derived by averaging the mean maximum and mean minimum temperatures for the locality and months concerned. Monthly mean maxima and minima for many Australian stations are given in the Council's Pamphlet No. 42.† There

* An officer of the Division of Food Preservation and Transport.

† "Meteorological Data for certain Australian Localities." Coun. Sci. Ind. Res. (Aust.), Pamph. No. 42 (1933).

are two assumptions implicit in this procedure. Firstly, it is assumed that the average of mean maximum and mean minimum is a good approximation to the arithmetic mean

$$\left(\frac{1}{T}\int_0^T \theta \, . \, dt\right).$$

Prescott* has investigated this point and he concludes that this approximation is generally very close. Secondly, it is assumed that the arithmetic mean temperature is a sufficiently close approximation to the effective mean temperature. The error introduced by this second assumption is discussed below.

The relation between the rate of many reactions and the temperature can be expressed to a high degree of accuracy by the equation

$$R = R_0 e^{b(\theta - \theta_0)} \qquad (1)$$

where R is the rate at temperature θ

R_0 is the rate at temperature θ_0

b is a constant characteristic of the reaction

and e has its usual meaning (2·71828 . . . . . .)

Diurnal fluctuations are generally the most important temperature changes to be considered, and as a first approximation these may be assumed to be sinusoidal

i.e., $\theta = \theta_0 + a \sin 2\pi t \qquad (2)$

where a is the amplitude of fluctuation (= half the range)

and t is the time in days from a suitably chosen zero.

Taking θ_0 as the zero of the temperature scale, the mean rate of reaction is given by

$$\overline{R} = \frac{R_0}{P}\int_0^P e^{b\theta} \, . \, dt$$

$$= \frac{R_0}{P}\int_0^P e^{ab \sin 2\pi t} \, . \, dt \qquad (3)$$

where P is a positive integer.

i.e., $$\frac{\overline{R}}{R_0} = I_0(ab) = 1 + \frac{(ab)^2}{2^2} + \frac{(ab)^4}{2^2 4^2} + \frac{(ab)^6}{2^2 4^2 6^2} + - - - - \qquad (4)$$

Writing $\overline{\theta}$ for the effective mean temperature

$$\frac{\overline{R}}{R_0} = e^{b\overline{\theta}}$$

$$= I_0(ab) \text{ from (1) and (4)}$$

$$\therefore \ \overline{\theta} = \frac{1}{b} \ln I_0(ab) \qquad (5)$$

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* Prescott, J. A.—*Trans. Roy. Soc., S. Aust.*, 58: 48 (1934).

The function $I_0(x)$ $[= J_0(ix)]$ is tabulated in various collections of tables and also in textbooks on Bessel functions. For small values of the argument it is easily calculated from the series. For large values of ab, θ tends to the value $a - \frac{1}{2b} \ln 2\pi ab$.

It is usual to specify the rate of change with temperature of reactions such as we are considering by means of the quantity Q_{10}.

By definition $Q_{10} = \frac{R_{\theta + 10}}{R_\theta}$

$= e^{10b}$ from (1)

$$\therefore b = \frac{1}{10} \ln Q_{10} \qquad (6)$$

temperatures being expressed in °C.

The values in Table 1 have been calculated from equations (5) and (6).

TABLE 1.—EXCESS OF EFFECTIVE MEAN TEMPERATURE OVER ARITHMETIC MEAN (°C.).

(Amplitude of sinusoidal fluctuation (= half the range) °C.)

Q_{10}.	0.	5.	10.	15.	20.
1·5	0·00	0·25	1·02	2·28	3·89
2	0·00	0·42	1·68	3·64	6·22
3	0·00	0·67	2·56	5·37	8·78
10	0·00	1·33	4·61	8·51	12·89

Most reactions in foodstuffs and living matter have Q_{10} values in the range 1·5 to 3. For the rate of killing of bacterial spores at sterilizing temperatures, a Q_{10} value of 10 is typical.

The appropriate value of the amplitude to use in applying the above table is, of course, that for the foodstuff and not that for the air temperature. For goods in stores the amplitude of fluctuation of foodstuff temperatures is generally much less than that of the air temperature, but goods exposed to the sun may show considerably greater variation. The yearly mean amplitude of diurnal fluctuation for a number of Australian localities is shown in Table 2.

TABLE 2.—YEARLY MEAN AMPLITUDE OF DIURNAL TEMPERATURE FLUCTUATION.

	Amplitude °C.		Amplitude °C.
Melbourne	4·9	Alice Springs	7·9
Sydney	3·9	Darwin	4·6
Brisbane	5·1	Hughenden	8·1
Cairns	4·6	Adelaide	5·4

It is evident from the above tables that for goods stored so that the direct effects of solar radiation are small, values of $\bar{\theta}$ will rarely exceed 1°C. in Australia and will generally be much less than this, i.e., the error involved in assuming the life will be equal to that of material held at the arithmetic mean temperature will be small.

Diurnal temperature fluctuations are never strictly sinusoidal in form, though those of goods in stacks are generally more nearly so than those of the surrounding air. Where the effects of solar radiation are large, the departures are likely to be particularly great. The effects of these departures from the simple theory can be determined by substituting the appropriate Fourier expansion for equation (2), but the series replacing that in equation (4) then becomes very cumbersome. With observational data it is simpler to evaluate

$$\frac{1}{P}\int_0^P e^{b\theta} \, . \, dt$$

by graphical integration.

This procedure was used to derive the "true" values given in Table 3. A Q_{10} value of 2·00 was assumed.

TABLE 3.—COMPARISON OF "TRUE" VALUES OF $\bar{\theta}$ WITH THOSE GIVEN BY THE SIMPLE THEORY FOR SOME OBSERVATIONAL DATA.

—	Range °C.	Arithmetic Mean Temperature °C.	Estimates of $\bar{\theta}$.	
			"True" Value.	Value Derived from Equation (5).
1. Air temperature at an Australian inland town	10·6 to 23·6	17·2	0·73	0·72
2. Air temperature immediately under a dark surface exposed to the sun in the same place	0·4 to 57·5	28·0	16·9	11·6
3. Temperature of top of can immediately below 2	12·5 to 42·2	25.7	4·96	3·59
4. Temperature of can of food exposed to the sun (air temperature range 25·3°–39·1°C.)	28·4 to 40·2	34·5	0·65	0·62
5. Temperature near the surface in a drum of dried apricot exposed to the sun (air temperature range 23·3°–32·8°C.)	22·4 to 56.5	32·9	4·62	4·67

These figures indicate that for surfaces exposed to solar radiation, equation (5) may give results significantly below the true value. Nevertheless it will probably be sufficiently accurate for most practical purposes.

The Determination of Carotene: A Critical Examination.

By C. R. Austin, M.Sc., B.V.Sc., A.A.C.I., and J. Shipton, B.Sc.Agr.**

Summary.

The investigation of problems encountered in developing an accurate and specific method for the estimation of carotene is described and discussed. The method itself may be outlined as follows.

The material is divided as finely as possible, and an amount containing 100 to 200 gamma of carotene is weighed into a flask. To this is added sufficient 20 per cent. aqueous KOH to cover the material, the volume of alkali used being noted. The mixture is brought to the boil on a hot-plate. After cooling, about six volumes of absolute ethyl alcohol are added and the mixture refluxed for 30 minutes. The extract is removed by filtration through a sintered glass funnel. The residue is triturated with alcohol and petroleum ether until no further colour can be extracted. An approximate account is kept of the alcohol used and, if necessary, the alcohol concentration of the filtrate adjusted so as to lie between 55 and 75 per cent. The solution is transferred to a separatory funnel and shaken vigorously. The petroleum layer is run into a flask and the alcoholic solution re-extracted with petroleum ether as a precautionary measure. The bulked petroleum extracts are now washed three times with equal volumes of water in a separatory funnel, run into a clean flask, and dried over anhydrous sodium sulphate.

The solution is now passed through a column of activated magnesium oxide and the carotene eluted with 10 per cent. acetone in petroleum ether. The eluate is made to a definite volume and the colour intensity measured with a suitable instrument.

1. Introduction.

Since the identification of the carotenes as Provitamins A, numerous methods have been proposed for the assay of these pigments in foods and other materials. No single method wholly satisfies the criteria of the ideal assay—accuracy, rapidity, and specificity—yet many fulfil the requirements of a particular part of the estimation. Thus, it appeared possible, by judicious selection from these methods, to evolve a process more closely approximating the ideal. It was with this object that the investigations discussed in this paper were undertaken.

The method to be described has, therefore, been subjected to thorough examination, and the necessary precautions have been clearly defined. The procedure, which has been successfully applied to a wide variety of foodstuffs, consists essentially of the following stages.

1. Extraction.
2. Removal of Non-Carotene Pigments.
3. Colour Measurement.

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* Assistant Research Officer, Division of Food Preservation and Transport.

2. Extraction.

The foodstuff should first be reduced to as finely divided a state as is conveniently possible: dried material may be passed through a C & N mill; fresh or moist materials may be sliced or minced.

Of the variety of methods designed for the extraction of carotene, that proposed by Seshan and Sen (1942) has been found the most generally satisfactory. This has been modified by the use of 20 per cent. aqueous KOH for the digestion of all types of foods, and by heating the mixture on a hot-plate so that it comes to the boil in about five minutes. Sufficient alkali is added to cover the sample. It is usually adequate to bring the mixture to the boil, though active boiling under reflux for two minutes is desirable for the thorough disruption of some tissues. Such treatment does not appear to result in any loss of carotene, even when boiling is continued for ten minutes. This is supported by tests in which a suspension of finely divided crystalline β-carotene in 20 per cent. KOH was boiled for periods up to 20 minutes. Slight loss was detected after 20 minutes boiling, but up to 10 minutes the recovery was complete.

Following the digestion with potash solution, six volumes of absolute ethyl alcohol are added and the mixture refluxed for 30 minutes. Exposure to window light (but not to sunlight) during this period has been found to cause no appreciable loss of carotene. If window light is excluded, the period of heating can be extended to two hours with impunity. In those methods which involve the extraction of the carotene from food by means of the Soxhlet extractor, it is advisable to shield the apparatus from direct window light. Owing to the length of time frequently required for complete extraction, losses of carotene approaching 10 per cent. have been observed, and these could be attributed to the influence of light.

The choice of the ratio of six volumes of alcohol to one of 20 per cent. aqueous KOH solution is based on the results illustrated in Fig. 1. These were derived from tests carried out on a variety of foods, which were heated with 20 per cent. aqueous KOH and then refluxed with alcohol added in the ratios indicated. The residue was removed by filtration and washed once with a small quantity of absolute ethyl alcohol, and the carotene content of the filtrate was determined (fraction 1). Any carotene remaining in the residue was removed by grinding in a mortar with alcohol and petroleum ether, and estimated (fraction 2). The sum of the values obtained for fractions 1 and 2 represents the carotene content of the original food sample. In Fig. 1 are shown the values of fraction 1 (as percentages of total carotene extracted) for each ratio of alcohol to alkali. In the case of ratios 6 : 1 and 7 : 1, fraction 2 contained no carotene. (However it should be clearly noted that, where a food cannot be reduced to a finely divided state, the residue will require further extraction, even though a ratio of 6 : 1 has been used.)

The Seshan and Sen method of extraction has been found decidedly more efficient than any of the commonly used methods of cold extraction, and more widely applicable than the process recommended by Peterson, Hughes, and Freeman (1937). Furthermore, in the latter, since the food is refluxed with 10 per cent. alcoholic KOH the alcohol

must first be freed of aldehydes which, otherwise, would form resins during the heating. These have a brown colour, and, being epiphasic to 90 per cent. methyl alcohol, would be measured as carotene if the partition technique were employed. With aqueous-alcoholic potash, no resinification occurs and thus no prior treatment of the alcohol is necessary.

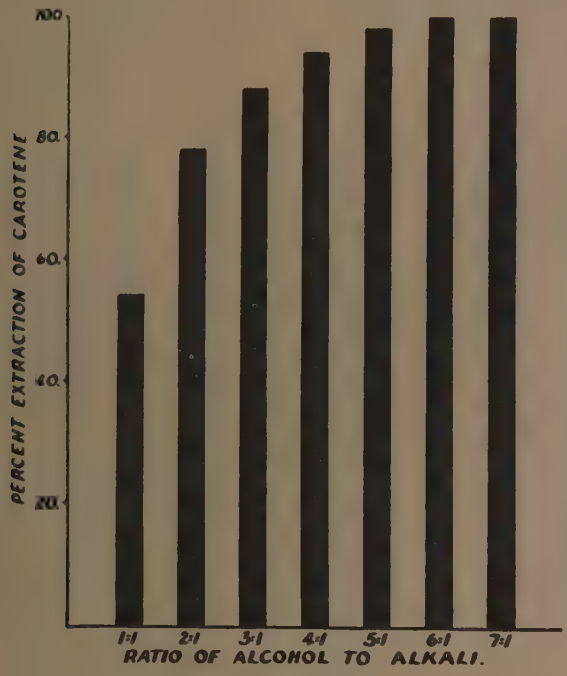

Fig. 1.—See text.

In a recent publication, Pepkowitz (1943) has shown that, when in solution, carotene undergoes some destruction in the presence of chlorophyll and light. In the absence of chlorophyll no such effect was observed. Since chlorophyll is rapidly destroyed by the alkali in the initial stages of the Seshan and Sen extraction, losses of carotene from this cause are avoided.

After refluxing, the alcoholic-alkaline digest is filtered through sintered glass. Relatively coarse filters, such as Jena 3G1 and 3G2 or Pyrex 2D1 and 2D2, are very useful for this purpose. The residue is stirred on the filter plate and washed with alcohol, which is then drawn through the filter. It is often advisable to transfer the residue to a mortar, where it is triturated with either absolute alcohol or petroleum ether, in order to check the completeness of the extraction. With a few foods of an unusually intractable nature (such as dried apricot and compressed fruit blocks) it may be found necessary, in some cases, to repeat the extraction.

The filtrate is now transferred to a separatory funnel and, if necessary, either alcohol or water added to adjust the alcohol concentration to between 55 per cent. and 75 per cent. (allowing for the moisture in fresh food material). This is most important, since the complete extraction of carotene by petroleum ether, which constitutes the next step, is difficult above this range and becomes impossible with alcohol concentrations below 55 per cent. In Fig. 2, there is illustrated graphically the influence of alcohol concentration upon the efficiency with which β-carotene may be extracted from a simple alcoholic solution.

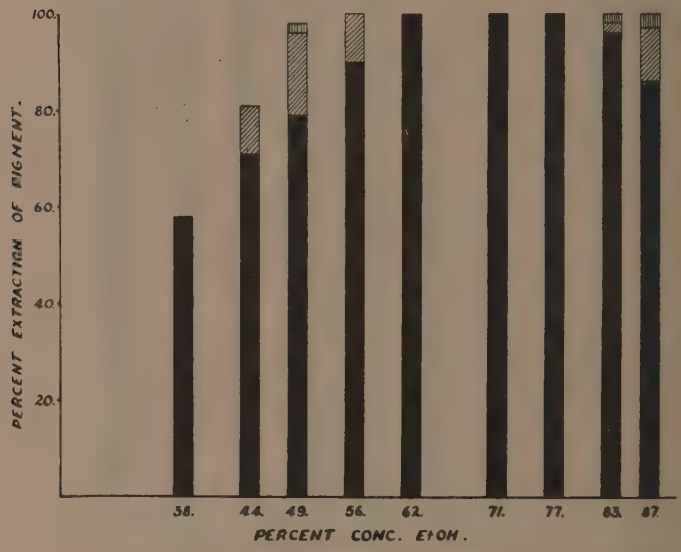

FIG. 2.—Effect of alcohol concentration on extraction of carotene with petroleum ether from simple alcoholic solution.

Black = first extraction; hatching = subsequent extractions.

In the case of a food extract the effect is even more marked (Fig. 3). For each of the concentrations indicated in these diagrams, extraction with petroleum ether was continued until no further pigment could be removed. Thus, in the case of carrot extract, at a concentration of 56 per cent., one extraction was sufficient to remove all the carotene, while at 44 per cent. two extractions removed only 56 per cent. of the carotene, and *it was not possible to remove any further carotene from this solution.* Below 40 per cent. alcohol, no further carotene could be removed after the first extraction. For this reason, and especially when assaying fresh material, it is advisable to use only absolute alcohol. It should be noted that the alcohol concentrations mentioned above are calculated on the basis of the amounts of alcohol and water involved and are, therefore, a little lower than the true concentrations as determined by measurement of specific gravity.

With the prescribed alcohol concentrations, a single extraction is sufficient to transfer all the carotene to the petroleum ether, though the process should be repeated as a precautionary measure. With some foods it will be found that colour still passes into the petroleum phase with three and four extractions. This colour is almost certainly not carotene, but may be tested by shaking the petroleum solution with 90 per cent. methyl alcohol. If the pigment is completely hypophasic it is of a non-carotene nature, and is usually classed as a "xanthophyll."

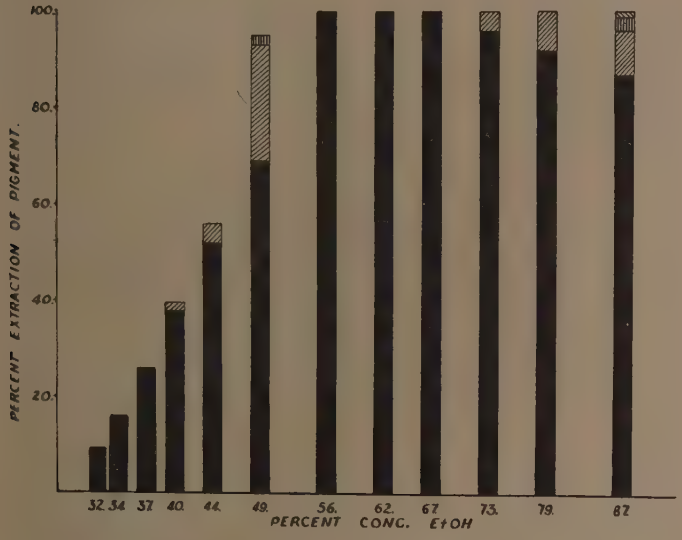

Fig. 3.—Effect of alcohol concentration on extraction of carotene with petroleum ether in the case of a food extract.

Black = first extraction; hatching = subsequent extractions.

3. Removal of Non-Carotene Pigments.

Two methods are available for the removal of non-carotene pigments. These are:—(*a*) partition, and (*b*) chromatography.

The relative merits of these two alternatives are now widely appreciated. It is more accurate and specific to employ a chromatogram, quite apart from the fact that it is not valid to assay certain foods (e.g. tomatoes, apricots, peaches, and rose hips) by the partition method because they contain large amounts of non-carotene epiphasic pigments such as lycopene. On the other hand, it may be desirable in some cases (e.g. yellow maize) to include cryptoxanthin in the assay because of its provitamin activity. Since this pigment is epiphasic to 90 per cent. methyl alcohol, it is estimated by the partition method, whereas it is normally omitted in routine chromatographic determinations.

(*A.*) *Partition.*

Classically this is carried out by means of 90 per cent. methyl alcohol, as proposed by Willstätter and Stoll (1913), and the description of the technique may be found in a number of publications (e.g. Seaber, 1940, and Gilman, 1938). In the experience of the present authors, the same effect may be achieved with 82 per cent. ethyl alcohol. This is demonstrated in Fig. 4, in which are recorded the partition coefficients of several concentrations of methyl and ethyl alcohols, for a variety of food extracts. The data show that the most efficient concentration of methyl alcohol for partitioning is between 90 per cent. and 92 per cent. and of ethyl alcohol between 82 per cent. and 84 per cent. Reference to the partition ratios (ordinates) shows that 90 per cent. methyl alcohol, as an extractant for non-carotene pigments, is only slightly more efficient than 82 per cent. ethyl alcohol.

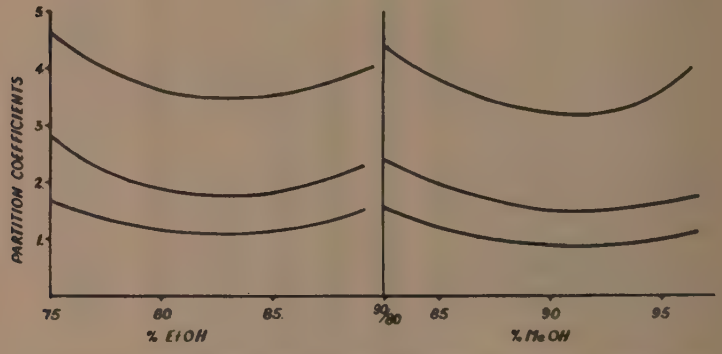

FIG. 4.—Partition coefficients between petroleum ether and various concentrations of ethyl and methyl alcohol of the pigments derived from three types of foodstuff. Upper curve is for fresh lucerne (high carotene to "xanthophyll" ratio), the middle curve is for lucerne chaff (containing altered pigments), and the lower curve is for fresh cabbage (low carotene to "xanthophyll" ratio).

Most of the pigments usually classed as "xanthophylls" readily pass into the hypophase with somewhat lower concentrations of alcohol than those used for the partition. Some, however, remain in the epiphase, and these include pigments usually encountered in dried foods, and often referred to as "oxidized carotenes." α-carotene, β-carotene, and lycopene remain in the epiphase with all alcohol concentrations investigated. Fig. 5 illustrates the behaviour of the "xanthophylls" from dried lucerne when the petroleum solution is exhaustively extracted with various concentrations of aqueous alcohol.

The inefficiency of the partition method in removing the non-carotene pigments is best expressed as a ratio between the figures obtained by the chromatographic and partition methods respectively.

Ratios for a variety of foods are recorded in Table 1. The similarity between 90 per cent. methyl alcohol and 82 per cent. ethyl alcohol is also apparent from these data.

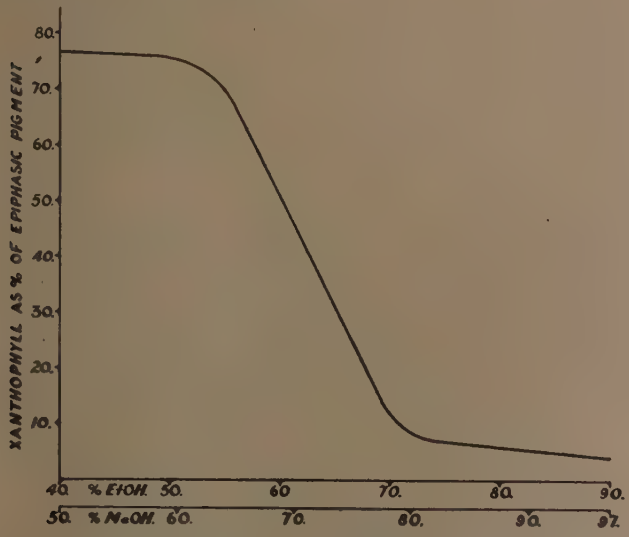

Fig. 5.—Behaviour of the "xanthophyll" fraction from dried lucerne when the petroleum solution is partitioned with various concentrations of aqueous alcohol.

Table 1.—Chromatographic to Partition Ratios.

Material.	90 Per Cent. MeOH.	82 Per Cent. EtOH.
Lettuce (*Lactuca sativa*)	1 : 1·06	1 : 1·11
French Bean (*Phaseolus vulgaris*) ..	1 : 1·12	1 : 1·15
Butter Bean (*Phaseolus vulgaris*) ..	1 : 1·20	1 : 1·23
Silver Beet (*Beta vulgaris*)	1 : 1·05	1 : 1·09
Cabbage (*Brassica oleracea*)	1 : 1·02	1 : 1·03
Parsley (*Carum petroselinum*)	1 : 1·10	1 : 1·09
Dried Parsley	1 : 1·13	1 : 1·13
Lucerne (*Medicago sativa*)	1 : 1·04	1 : 1·07
Dried Lucerne	1 : 1·15	1 : 1·15
Carrot (*Daucus carota*)	1 : 1·03	1 : 1·04
Dried Carrot	1 : 1·10	1 : 1·11
Pumpkin Fruit (*Cucurbita pepo*)	1 : 1·30	1 : 1·30
Maize Grain (*Zea mays*)	1 : 2·82	1 : 2·82

(*B.*) *Chromatography.*

By the chromatographic method the complete removal of extraneous pigments is easy and certain, and it is unquestionably the method of choice. In this connection it is necessary to consider:—(i) the adsorbent, (ii) the solvent, (iii) the preparation of the column, and (iv) the adsorption of pigments and elution of carotene.

(*i*) *The Adsorbent.*

Several adsorbents (alumina, magnesia, calcium hydroxide, calcium carbonate, and dicalcium phosphate) have been recommended for assay work with carotene. In this laboratory, magnesium oxide has been found the most satisfactory. There are a number of proprietary forms of this material on the market, differing from one another in their particle size, adsorptive power, and tendency to become destructive of carotene.

The criteria which indicate the suitability of a magnesia for routine assay work are:—(*a*) strong adsorptive power, (*b*) rapid rate of filtration, (*c*) facility of packing into a column, and (*d*) freedom from any destructive tendency.

(*a*) *Adsorptive Power.* In general, the smaller the particle size the stronger the adsorptive power, although the latter is more dependent on the mode of preparation of the magnesia than upon its particle size. The most suitable material is prepared by the dehydration of magnesium hydroxide (Zechmeister and Cholnoky, 1941).

As a measure of adsorptive power, LeRosen (1942) has introduced the term R, which is the ratio of the rate of movement of the solvent through the column to the rate of movement of an adsorbed pigment band. With β-carotene as the adsorbed band and petroleum ether as the solvent, a suitable magnesia may be defined as one with which the value of R is not less than 60.

The following brands of magnesia, after activation, have been found to give a value of R in excess of 80: B.D.H. ("Heavy"), Hopkins and Williams ("Heavy"), and Howard and Co. ("Heavy"). These are the only brands of "Heavy" magnesias that have been tested and it is probable that others may be equally suitable. With "Micron" brand magnesia, mixed in equal parts with "Hyflo Super Cel", R approaches 300 when the magnesia is activated, and 100 when not activated. When mixed in the proportion of 1 : 3 the values of R are 100 and 30 respectively. "Micron" magnesia is too fine a powder to be used without a filter aid.

To render weak pigment bands readily distinguishable, the column should be quite white. The "Heavy" magnesias mentioned above fulfil this condition, but "Micron" has a slight grey-brown colour when wet with the solvent.

(*b*) *Rate of Filtration.* For the rapid determination of carotene, the rate of flow of solvent through the adsorbent should not be less than 50 to 60 mm./min.

This is equivalent, in the case of a column of 2·5 cm. diameter, to a filtration rate of 20 ml./min., and is readily obtained with the coarser magnesias with the help of a suction pump.

With "Micron" brand magnesia, even when mixed with a filter-aid, only slow speeds can be attained by this means. Using columns 2·5 cm. by 9 cm., the maximum filtration rates that could be obtained were as follows:—

B.D.H. ("Heavy")	28ml./min.
"Micron" (1 part to 1 part filter-aid)	5ml./min.
"Micron" (1 part to 3 parts filter-aid)	14ml./min.

(*c*) *Ease of Packing.* In general, the coarser the magnesia the more easily may it be packed into a compact column. With the "Heavy" magnesias no difficulty is encountered with tubes up to 5 cm. in diameter. On the other hand, the preparation of satisfactory columns from "Micron" magnesia mixtures requires rather more specialized technique (*vide infra*).

(*d*) *Destructive Tendencies.* Destruction, or loss, of carotene occurs when the pigment is adsorbed on the column and is very marked with certain varieties of magnesia. By destruction is implied the appearance of an obviously discoloured zone in the position previously occupied by the pigment, after the latter has been moved downward (by development or elution). It should, however, be noted that appreciable destruction occurs only when relatively concentrated solutions of pigments (e.g., of the order of 200 mgm. to 300 mgm. of β-carotene per 100 ml.) are being passed through the column. Furthermore, magnesias appear to differ in their proneness to become destructive. Thus, B.D.H. ("Heavy") magnesia becomes slightly destructive if activated at 120°C., more so at 250°C., and markedly so at 500°C. On the other hand, Hopkins and Williams ("Heavy"), Howard and Co. ("Heavy"), and "Micron" magnesias exhibit no apparent destructive properties until heated above 250°C., and even after activation at 500°C. do so to only a small extent.

With the brands of magnesia mentioned this destructive effect is not of much practical importance. In an assay, the concentration of carotene should not exceed 300 to 400 gamma per 100 ml., and solutions containing up to 5,000 gamma per 100 ml. have been passed through columns of known destructive power without apparent loss. This permits the recovery of these magnesias by heating to 500°C. in a furnace. The possibility that some brands of magnesia may develop a destructive propensity which is of practical significance should, however, be borne in mind.

(ii) *The Solvent.*

Petroleum ether (boiling range 60° to 80°C.) forms a useful solvent for chromatography, though a lighter fraction (boiling range 30° to 60°C.) allows slightly better adsorption. In the writers' experience, most of the commercial grades of petroleum ether, though colourless themselves, contain substances which produce pigment-like bands on magnesia columns. Such solvents should, therefore, always be treated by passage through activated magnesia before being used for chromatography.

Water is very slightly soluble in petroleum ether (about 0·3 per cent.) and unless removed it has a somewhat detrimental effect upon the adsorption. For example, in one test R (defined above) was 68 with dry solvent and 55 with water-saturated (though perfectly clear) solvent. For this reason the carotene-containing solution should be dried over anhydrous sodium sulphate or calcium chloride before being passed through the column.

The petroleum extract intended for chromatography should be quite free of alcohol, since this has a very marked effect on the adsorption of carotene. As little as 0·01 per cent. of alcohol in the extract will cause a fourfold increase in the rate of movement of β-carotene (see

Fig. 6). Therefore, after extraction of the alcoholic-alkaline digest of a food with petroleum ether, the latter should be washed at least twice with an equal volume of water.

(iii) *Preparation of the Column.*

For assay work, apparatus such as that illustrated by Morton (1942) is used. The packing of such an adsorption tube with the coarser magnesias is not a difficult matter. A plug of cotton wool is pushed down to the end of the wider part of the tube and the necessary quantity of magnesia is poured in through a funnel. The actual packing

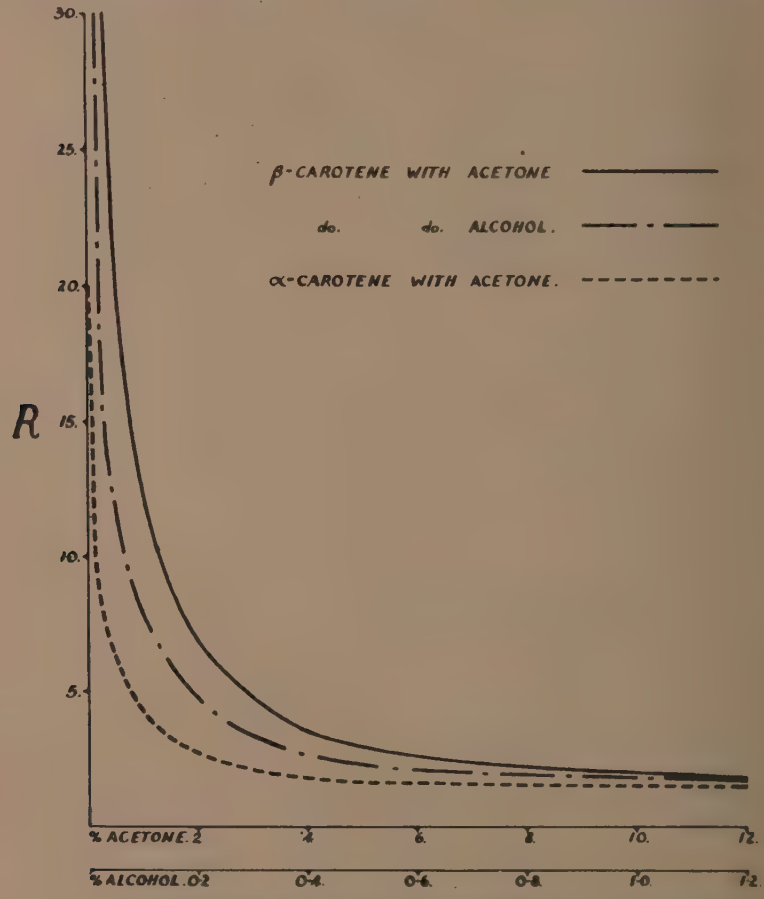

Fig. 6.—Illustrating the influence of various concentrations of acetone and alcohol on the rates of movement of α- and β-carotene in a chromatogram. (Adsorbent — magnesium oxide).

is done by dropping the tube rapidly some thirty or forty times on to a bench from a height of two to three inches. The tube is then connected to the pressure flask, and with gentle application of suction

from the water pump the solvent is allowed to drop slowly on to the column until about one inch of the adsorbent is saturated. A small quantity of solvent is then allowed to accumulate on the top of the column. Full suction may now be applied, taking care not to draw any air through the magnesia. The entire column should be saturated before the test solution is added. This method of packing has given satisfactory results with columns from 0·5 cm. to 5 cm. in diameter.

In the case of fine adsorbents, such as "Micron" brand, rather more care is necessary, and the method described by Wall and Kelley (1943) should be adopted.

(iv) *Adsorption of Pigments and Elution of Carotene.*

After the column has been washed with petroleum ether, the pigment solution is drawn through as recommended by Wall and Kelley—as rapidly as possible, with the aid of a suction-pump. The speed of filtration has little or no influence on the efficiency of adsorption (see Table 2).

Table 2.—Showing That R is not Influenced by the Rate of Filtration.

Rate of Filtration.	Concentrations of Acetone in Solution.	R.
mm./min.	%	
9·8	nil	64
35·0	nil	72
8·4	0·3	55
23·0	0·3	51
36·0	0·3	57
2·6	0·7	13
71·0	0·7	14
6·0	3·6	4·7
18·0	3·6	5·0
31·0	3·6	4·6

After all the pigment has been adsorbed the carotene may be eluted from the column. Occasionally it is desirable to elute α-carotene and β-carotene separately. For these purposes, solutions of acetone or alcohol in petroleum ether may be used, the former being preferred. The appropriate concentrations of these solvents will depend upon (*a*) the pigment to be eluted and (*b*) the activity of the magnesia. As a general guide to the selection of a suitable concentration, Fig. 6 is inserted.

It should be noted that the value of R with low concentrations of acetone (up to 1 per cent.) and alcohol (up to 0·05 per cent.) depends as much on the activity of the magnesia as upon the composition of the eluent mixture.

The data recorded in Fig. 6 also show that:—

(*a*) Alcohol is a little more than ten times as effective an eluent as acetone.

(*b*) The most efficient concentration of acetone for eluting β-carotene is about 10 per cent., whilst for α-carotene it is about 4 per cent.

(*c*) The most suitable concentration of acetone for separating α-carotene and β-carotene is in the region of 1 per cent.

4. Colour Measurement.

The colour of the carotene solution may be measured in almost any of the numerous photometric instruments on the market. If a spectrophotometer is not available, the instrument used should be fitted with a filter of a relatively narrow range of transmission. The maximum transmission should preferably correspond to the principal absorption maximum of β-carotene, viz., 452 mμ (in petroleum). If the instrument has been calibrated with pure β-carotene, an error will be involved in measuring solutions containing a proportion of α-carotene. However, this error is without practical significance for the following reasons:—(i) the absorption curves of α- and β-carotene are, in general, very similar; (ii) even the best filters have a relatively wide range of transmission (so that differences between the light absorption of the two pigments over a small part of spectrum would not be appreciated), and (iii) according to Rosenberg (1942), α-carotene is not known to amount to more than 40 per cent. of the total carotene present in foods; in most cases it does not exceed 10 per cent.

5. Acknowledgments.

The authors wish to record their gratitude to Mr. E. Parrish for the photographic reproduction of the figures and to Dr. M. C. Franklin for helpful criticism and advice on the preparation of the paper.

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Automatic Timing of Exposures for Photographic Printing.

*By A. A. Townsend, M.Sc.**

Summary.

An automatic timer has been built in the laboratory for use by the Photographic Section. It has proved of value in simplifying the production of a number of prints from the same negative. Since the construction is very simple and it is thought that some photographic studios may find the device useful, a description is given of the timer. The range is from approximately 0·3 second to 30 seconds, and may easily be extended in the upward direction. The switching capacity is approximately 300 watts, but this could be increased by making use of a more suitable relay.

1. Introduction.

Repetition printing of photographs is a process often carried out by counting seconds during the exposure. This process is reasonably efficient, when exposures of order of five seconds are involved, but, particularly, for contact printing, exposures under two seconds are often used. Then, it is extremely difficult to avoid considerable variations in exposure time, and the value of an automatic timer is very apparent. As the work in the photographic studio of the Division involves frequent reproduction of up to 50 prints, a simple electronic timer has been developed for use with the contact printing cabinet. This has proved completely satisfactory in operation and occupies very little space.

2. General Description.

The timing circuit is housed in a metal box, measuring 6½ inches by 9 inches by 4 inches. The operating switch is a two-way switch, originally intended for use with hand switching, and mounted on the cabinet, but it could as conveniently be mounted on the timing unit. Two controls are provided: a five-position switch giving four ranges and a position for manual control, and a potentiometer giving a fine control over the exposure time. Exposure time from 0·3 second to slightly over 30 seconds are available. The lower limit is set by the time taken by the relay to drop out, and could be decreased if necessary. To enable exposure times to be reproduced, circular scales are engraved on the panel.

3. Circuit.

The timer depends for its operation on the time taken for a condenser to discharge through a resistance. When the switch is in the off position, the condenser is charged to —150 volts. On operation of the switch, the condenser is connected to the grid of the relay pentode, and commences to discharge through the grid leak. The application of —150V to the grid cuts off the current of the pentode, thus releasing the relay and switching on the lights. The relay does not close until the condenser has discharged sufficiently to allow enough current to pass through the relay to operate it.

* An officer of the Division of Aeronautics.

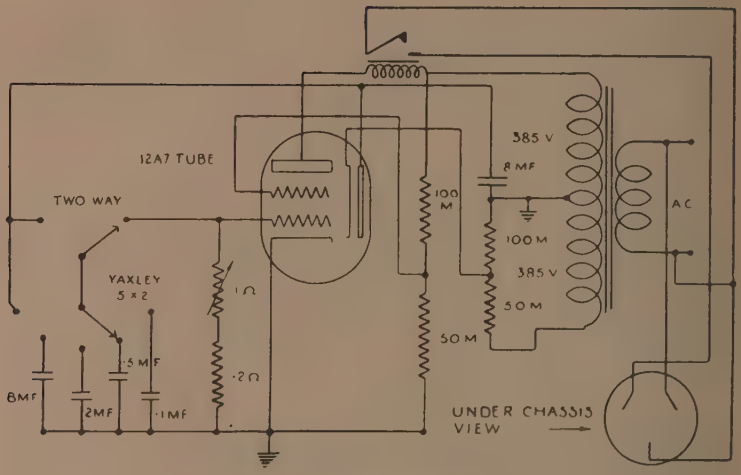

FIG. 1.—Circuit Diagram.

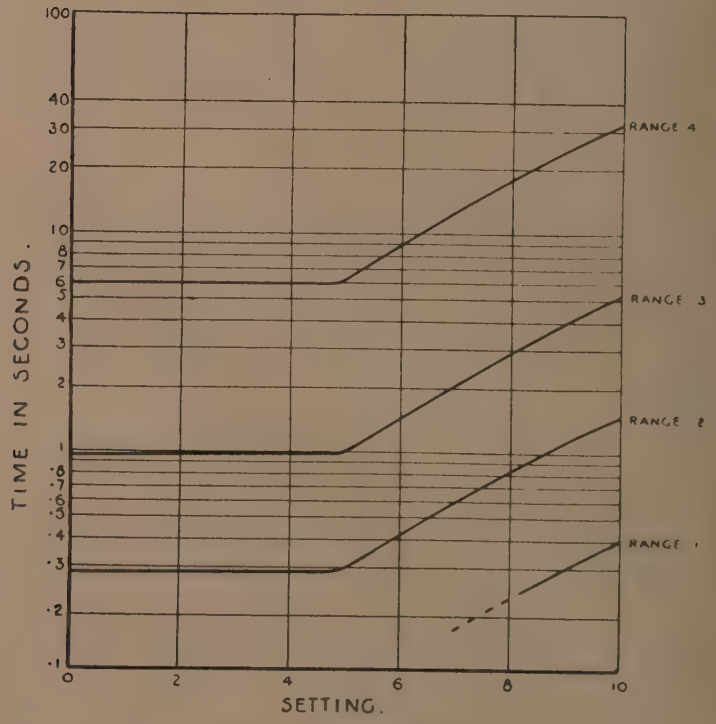

FIG. 2.—Calibration Curves.

The circuit employed is shown in detail in Fig. 1. It will be noted that a type 12A7 valve is used. This valve was selected as it can be used both as a relay and a bias rectifier. This made for a compact assembly, but it is probable that valves of local manufacture could be substituted. The combination of a 6J7–G and a 6X5–GT would certainly work, while a 6B8–G might be used with minor changes in the circuit.

4. Performance.

Curves illustrating the range of exposure times available are given in Fig. 2. The times appear to be consistent to within a few per cent., i.e., no deviations have been reliably detected below a time of ten seconds. Calibration curves are shown in Fig. 2. As can be seen, nearly all the variation occurs over the top section of the potentiometer control. This is due to the use of the standard resistance taper.

Fig. 3.—Automatic timer installed on printing cabinet.

The range is from about 0·3 second to slightly over 30 seconds, but there would be little difficulty in extending the range upwards. Shorter intervals than 0·3 second could be obtained by substituting a relay with faster release characteristics, but they are not usually necessary. The switching capacity is approximately 300 watts, which could be increased, if tungsten contacts were employed on the relay.

The apparatus is self-contained and requires no particular adjustment to make it work. It is shown mounted on the printing cabinet in Fig. 3.

NOTES.

Retirement of Mr. I. H. Boas from Division of Forest Products.

Mr. I. H. Boas retired from the position of Chief of the Division of Forest Products at the end of March, and he was succeeded by Mr. S. A. Clarke, formerly Deputy-Chief.

Mr. Boas has been Chief of the Division since its establishment in 1928, and under his leadership the Division has become well known in every State of the Commonwealth and has been able to render great assistance to the timber trade and timber users in general. Mr. Boas interested himself in forest products long before he became associated with the Council. For instance, in 1928, while in Western Australia, he proved that it was possible to produce paper from Australian hardwoods despite advice to the contrary from authorities used to Northern Hemisphere timbers. Following intensive work by others, the recent establishment of valuable pulp and paper mills has resulted.

On his retirement, the Division of Forest Products presented Mr. Boas with a cabinet made of Australian timbers, and a composite photograph showing members of the staff. The Division of Aeronautics presented him with a moulded plywood tray made from scented satinwood and faced with figured Queensland maple. Mr. Boas is not completely severing his association with the Division of Forest Products, for he has consented to remain as a part-time consultant.

The Preparation of Sections of Copper-Lead Alloys for Metallographic Examination.

A paper of the above title by R. W. K. Honeycombe, an officer of the Lubricants and Bearings Section of the Council, will appear shortly in the Proceedings of the Australian Institute of Mining and Metallurgy.

The paper describes a method for polishing copper-lead bearing alloys for micro-examination, and deals briefly with metallographic characteristics of these alloys, and the detection of defects frequently encountered. A number of typical micro-structures are shown.

Review.

"A Dictionary of the Fungi," by G. C. Ainsworth and G. R. Bisby.

(Published by the Imperial Mycological Institute, Ferry Lane, Kew, Surrey, England, 1943, pp. viii + 359, plates 10. Price 20s. sterling, post free.)

The authors and the Imperial Mycological Institute are to be congratulated on the production of their dictionary of the fungi. To a mycologist it is at once evident that an onerous and difficult task has been ably accomplished, with the result that a most valuable compact compendium is available to mycological workers. I would prefer the explanatory notes at the foot of page 117 to be on page 1 with the notes at the beginning of the work. It seems a pity that the cover is dark-blue with black printing, so that the title of this excellent work is barely visible.

—B. T. Dickson.

Recent Publications of the Council.

Since the last issue of this *Journal*, the following publications of the Council have been issued:—

Bulletin No. 172.—"Zebu-Cross Cattle in Northern Australia. An Ecological Experiment," by R. B. Kelley, D.V.Sc.

This Bulletin gives the history of the importation of Zebus into Australia, and describes the results of the experiments up to date. Although a few Zebu cattle had been brought into Australia previously, the first systematic importation was in 1933. In that year the Council for Scientific and Industrial Research, in co-operation with several pastoralists, imported 18 Zebu cattle and one cross-bred from America and settled them on four properties of co-operating pastoralists in different parts of Queensland. Under the supervision of the Council, systematic cross-breeding with British breeds was undertaken, until in 1941 there were over 8,000 Zebu-cross cattle, mostly quarter-breds.

The two main reasons why British breeds are not at their best in the tropical areas of Australia, are that their body temperatures rise above normal levels in hot weather, and they suffer badly from "tick-worry." Zebus, on the other hand, suffer no discomfort in hot weather, and the pure-bred animals are completely tick-repellent. Half-bred Zebus, though harbouring a few ticks, rarely show "tick-worry," but the susceptibility of quarter-breds is variable, depending upon their type of coat. Zebu-cross cattle also appear to be more tolerant of drought conditions. It has been found that they reach a marketable size about a year earlier than British breeds, possibly because they can thrive during the dry season. The carcases of the cross-breds compare favourably with those of British breeds reared in the same areas.

Bulletin No. 174.—"Recent Advances in the Prevention and Treatment of Blowfly Strike in Sheep," Supplement to Report No. 2 by the Joint Blowfly Committee.

The Joint Blowfly Committee co-ordinates the sheep blowfly research undertaken by the Council, the New South Wales Department of Agriculture, and the Queensland Department of Agriculture and Stock.

The report is in the nature of a practical handbook, and it is being distributed mainly by the various State Departments of Agriculture. It is designed to bring Report No. 2 (Pamphlet 98 in the Council's series) up to date, but it may also be issued in place of that Report, which is now almost out of print.

Since the issue of Pamphlet 98, the Mules operation has been modified and rendered much more effective; the new technique is described in the present Bulletin. Some confusion had arisen over the correct length to dock the tails of lambs, and this is now clarified. Finally, full details are given for the preparation of B.T.B. and B.K.B. dressings for the treatment of strike; these have both been evolved since the issue of the last report.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. 175.—"The Recovery of Inter-block Information in Quasi-Factorial Designs with Incomplete Data. 2. Lattice Squares," by E. A. Cornish, M.Sc., B.Agr.Sc.

Bulletin No. 176.—"The Analysis of Cubic Lattice Designs in Varietal Trials," by I. F. Phipps, M.Sc., B.Agr.Sc., Ph.D., A. T. Pugsley, B.Agr.Sc., S. R. Hockley, and E. A. Cornish, M.Sc., B.Agr.Sc.

Bulletin No. 177.—"A Soil Map of Australia," by J. A. Prescott, D.Sc., A.A.C.I.

Bulletin No. 178.—"Food Composition Tables," compiled by Hedley R. Marston and Mary C. Dawbarn.

Bulletin No. 179.—"Lubrication between the Piston Rings and Cylinder Wall of a Running Engine," by J. S. Courtney-Pratt, B.E., and G. K. Tudor, B.E.

Bulletin No. .—"Studies on Deglutition in Sheep. 1.—Observations on the Course Taken by Liquids through the Stomach of the Sheep at Various Ages from Birth to Maturity," by R. H. Watson, D.Agr.Sc. "2.—Observations on the Influence of Copper Salts on the Course Taken by Liquids into the Stomach of the Sheep," by R. H. Watson, D.Agr.Sc., and I. G. Jarrett, B.Sc.

Bulletin No. .—"Sheep Blowfly Investigations. The Attractiveness of Sheep for *Lucilia cuprina*," by I. M. Mackerras, M.B., Ch.M., B.Sc., and M. J. Mackerras, M.B., M.Sc.

Bulletin No. .—"The Effectiveness of Various Mineral Dusts for the Control of Grain Pests," by J. S. Fitzgerald, M.Sc., Ph.D., A.A.C.I.

H. E. Daw, Government Printer, Melbourne.

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